Neurophysin Biosynthesis In Vitro in Oat Cell Carcinoma of the Lung with Ectopic Vasopressin Production

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A B S T R A C T The incorporation of labeled compounds into neurophysins of a transplantable human oat cell carcinoma of the lung with ectopic vasopressin production was studied in vitro. Neurophysins in cell extracts and in incubation media were isolated by immunoprecipitation and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. When cells were incubated with L-[³⁵S]cysteine for 12 h, SDS-polyacrylamide gel electrophoresis of the immunoprecipitates from cell extract and medium resolved two forms of neurophysins with apparent molecular mass of 10,000 (10K) and 20,000 (20K). Both forms of [³⁵S]-neurophysins were completely displaced from the immunoprecipitates by excess human neurophysin. Incubation of cells with L-[³⁵S]cysteine and D-[³H]-glucosamine hydrochloride revealed that glucosamine was incorporated into the 20K neurophysin region, but not into 10K species. To observe the kinetics of labeling of the two forms of neurophysins, cells were incubated with L-[³⁵S]cysteine for varying periods of time. After short labeling periods, most of the radioactivity resided in 20K species, which plateaued after 1 h, whereas 10K neurophysin progressively increased in its height. When cells were chased with unlabeled cysteine after the exposure to a short pulse of labeling, 20K neurophysin peak gradually decreased with an apparent initial half-life of 1 h. In contrast, the label in 10K neurophysin steadily increased, which exceeded the former by 3 h of chase. Analysis of 20K neurophysin in cell extract by isoelectric focusing on polyacrylamide gel demonstrated that it was principally composed of a protein with an apparent isoelectric point (pI) of 5.7. These results suggest that neurophysin is synthesized in ectopic vasopressin-producing tumors by post-translational processing from a glycosylated pro-neurophysin with an apparent molecular mass of 20,000 daltons and a pI of 5.7.

INTRODUCTION

Neurophysins are known to be closely associated with vasopressin and oxytocin (1). They appear to be biosynthesized concomitantly with the hormones in the hypothalamus and to be secreted into the circulation in response to stimuli that may elicit the release of vasopressin and oxytocin. Although the association of malignant tumor and inappropriate secretion of antidiuretic hormone (ADH) is now recognized frequently and tumor production of vasopressin may be one of the principal causes for inappropriate ADH syndrome in such patients, whether vasopressin coexists with its neurophysin in these tumors has been controversial (2-4).

We have recently shown that both immunoreactive vasopressin and neurophysins are unequivocally detectable in oat cell carcinomas of the lung with ectopic vasopressin production (5). When the tissue extracts were analyzed by gel filtration combined with neurophysin radioimmunoassay, a form of neurophysin with a high molecular mass of 20,000 daltons was consistently demonstrated. Based on the observation that tryptic digestion under controlled conditions generated an equimolar amount of vasopressin and neurophysin, we have postulated that

This study was presented in part at the Sixth International Congress of Endocrinology, Melbourne, Australia, 10-16 February 1980.
Received for publication 4 May 1981 and in revised form 22 July 1981.

1Abbreviations used in this paper: ADH, anti-diuretic hormone; ESN, estrogen-stimulated neurophysin; NIH, National Institutes of Health, NSN, nicotine-stimulated neurophysin; PBS, phosphate-buffered saline; pl, isoelectric point; SDS, sodium dodecyl sulfate; 10K, 10,000; 20K, 20,000.
this 20,000-dalton neurophysin is a common precursor to vasopressin and neurophysin (5). To further establish that 20,000-dalton neurophysin is proneurophysin, the kinetics of labeling of neurophysins were examined using vasopressin-producing oat cell tumor tissues transplanted into nude mice.

**METHODS**

**Tumor tissues.** Tumor tissue obtained at autopsy from a 58-yr-old man with oat cell carcinoma of the lung who developed inappropriate ADH secretion was transplanted into nude mice. The details of the method of transplantation as well as the results of the transplantation study in tumor-bearing animals were described elsewhere (6). The tissues contained a significant amount of both nicotine-stimulated neurophysin (NSN) (7) (2.873 ± .458 ng/g tissue, mean ± SEM, n = 6) and vasopressin (206 ± 78 ng/g tissue, mean ± SEM, n = 4) determined by radioimmunoassays, although the tissues were obtained from different animals at different passages. Estrogen-stimulated neurophysin (ESN) (8) was also detected in these tissues, however, the levels were low compared with NSN, that is, <1% of NSN. Similarly, the tumor content of oxytocin was minute corresponding to <1% of that of vasopressin.

**Purification of human posterior pituitary neurophysin.** Neurophysins were extracted from 17 frozen human posterior pituitary glands by the method of Hollenberg and Hope (8) and fractionated by a column (2.6 x 100 cm) of Sephadex G-50 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Upssala, Sweden), which was equilibrated and eluted with 0.1 N formic acid. Immunoreactive NSN and ESN were localized by diluting a portion of each fraction and carrying out radiolmmunoassays. Fractions containing immunoreactive NSN were pooled and rechromatographed on a column (1 x 50 cm) of Sephadex G-50 superfine followed by elution with 0.2 N acetic acid. The fractions that constituted the descending limb of the NSN peak were then combined and subjected to electrophoresis. Electrophoresis was performed on a column (1.7 x 35 cm) of Biogel P-2 (Bio-Rad Laboratories, Richmond, Calif.), 200-400 mesh, equilibrated with 0.05 M L-carnosine (pH 8.6) at a current of 6.6 mA/cm² for 20 h (5). A peak of immunoreactive NSN was observed in the α-globulin region, and the NSN fractions devoid of NSN activity were pooled and lyophilized. Approximately 1 mg of protein was recovered. To test the purity of the NSN preparation obtained, a portion was subjected to polyacrylamide gel electrophoresis using a 7-cm separating gel of 7.5% acrylamide and a 1-cm stacking gel of 3% acrylamide, both in Tris-glycine buffer (pH 8.5). A single protein band was obtained, while crude neurophysin preparation eluted from large Sephadex G-50 column showed three major bands. Judging from its migration pattern, the NSN thus purified appears to correspond to FC II of Robinson (7).

**Radioimmunoassay.** NSN and ESN concentrations in resulting fractions from exclusion chromatography and column electrophoresis as well as in tumor tissue extracts were estimated by radioimmunoassays (7) using immunological materials kindly donated by the National Institute of Arthritis, Metabolism and Digestive Diseases and the National Pituitary Agency, U. S. Public Health Service. General procedures of neurophysin radioimmunoassay were described in detail elsewhere (9). Vasopressin and oxytocin were radioimmunoassayed as previously reported (5, 10).

**Antisera for immunoprecipitation.** In initial studies, anti-NSN serum distributed by the National Institute of Arthritis, Metabolism and Digestive Diseases and the National Pituitary Agency, U. S. Public Health Service was used for the immunoprecipitation of neurophysins in cell extracts as well as in incubation media. Because of the limited supply of the antisera, however, we were obliged to search for an alternate. At that time, we have found that sera from patients with diabetes insipidus treated with vasopressin taken in oil (Pitressin tannate, Parke-Davis, Morris Plains, N. J.) showed a significant binding of 125I-ESN as well as 125I-NSN, which suggests that the patients were immunized with bovine or porcine neurophysin contaminated in the vasopressin tannate preparation (11). In Fig. 1 is shown the dose-related binding of labeled NSN and ESN (10 pg) to sera from six patients who have received vasopressin tannate. All of the sera bound 125I-labeled NSN significantly and the dose-response curves constructed with varying volumes of the sera were of similar shape and slope (Fig. 1A). Binding of 125I-labeled ESN was also demonstrated in five of the six sera, although larger volumes of sera were required (Fig. 1B). The possibility that the serum from the patient 1 with the highest titer for NSN could be useful for the purpose of immunoprecipitation was then examined.

**Purification of the antisera for immunoprecipitation.** Human neurophysin solution isolated as described above was covalently linked to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals) according to the method of Cuatrecasas (12). The affinity resin contained 0.8 mg of protein/ml of resin. The resulting neurophysin-Sepharose complex was packed in a 1 ml column at 4°C, and extensively washed with 0.1 M borate buffer (pH 8.5) and 0.1 M acetate buffer (pH 4.0). Immediately before use, the column was prewashed with 4 ml of 0.1 M guanidine hydrochloride, 10 ml of 0.01 M phosphate buffer—0.14 M sodium chloride, pH 7.0, containing 0.1% sodium azide (PBS) and 2 ml of normal human serum. 3 ml of the serum from the patient 1 diluted three fold with PBS was applied to the column and further washed with 16 ml of PBS, 10 ml of 1 M guanidine hydrochloride and 10 ml of PBS followed by elution with 10 ml of 4.5 M MgCl₂. The eluate was repeatedly dialyzed against PBS and concentrated to the original volume by use of Diaflo ultrafiltration membrane PM-10 (Amicon Corp., Scientific Sys. Div., Lexington, Mass.). Approximately 60% of the binding capacity for 125I-labeled NSN in crude serum was recovered after purification.

**Incubation studies.** Tumor tissues were aseptically excised from tumor-bearing nude mice and dispersed cells were prepared using trypsin and collagenase (13). Cells were suspended in Minimum Essential Medium in Earle’s solution (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) containing 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate. 2-ml aliquot containing ~100,000 cells was plated in each plastic petri dish (35 x 10 mm) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Viability of tumor cells at plating, determined by trypsin blue exclusion, was >90%.

After overnight incubation, the culture medium containing tumor cells was collected and centrifuged at 150 g for 10 min. The supernate was decanted and the cell pellet was washed once with cysteine-free culture medium. Cells were resuspended in cysteine-free medium and 2-ml aliquot was plated in each petri dish. After incubation at 37°C for 4–5 h under the atmosphere of 95% air-5% CO₂, 50 μCi of L-[3H]cysteine (470–1,350 Ci/mmol, New England Nuclear, Boston, Mass.) was added to the medium and incubation was continued for varying periods. In some experiments, cells were coincubated with D-6-[3H]glucosamine hydrochloride (20–30 Ci/mmol, Radiochemical Centre, London, England). For pulse-labeling experiment, 1.2 mg of unlabeled L-cysteine was added to the incubation medium after the labeling period.
of 30 min and cells were harvested after varying periods of incubation. At the end of the incubation, the medium was removed and centrifuged at 4°C. Cell pellet and medium were separated, immediately frozen on dry ice and kept at -70°C until analyzed.

**Extraction and immunoprecipitation.** Extraction and immunoprecipitation of neurophysins in cell extracts were conducted according to the general method described by Mains and Epper for ACTH (14) with minor modifications. Cells were extracted into 0.5 ml of 0.1 N HCl containing 5 mg/ml of bovine serum albumin and 1% solution of 30 mg/ml of phenylmethylsulfonyl fluoride/30 mg/ml of iodoacetamide in ethanol. Samples were frozen and thawed several times on dry ice, and homogenized with a Teflon pestle. Extracts were centrifuged at 2,300 g for 15 min at 4°C and the supernate was lyophilized. The residue was dissolved in 50–100 μl of 0.01 M phosphate buffer containing 0.1 M Na2EDTA and 0.1% Triton X-100 (pH 7.6) followed by titration to pH 7.0 with 0.1 N NaOH. After centrifugation, an aliquot of the supernatant was incubated at 4°C for 24 h with 5–20 μl of the purified human antineurophysin serum or 100 μl of anti-NSN serum provided by National Institutes of Health (NIH) at 1:200 dilution. As a control, 5 μg of purified human neurophysin was added to the tube. A sufficient amount of IgG fraction of anti-human IgG (Miles Laboratories, Elkhart, Ind.) or anti-rabbit IgG was then added and incubation was continued overnight. Incubation was terminated by adding 2 ml of 0.05 M phosphe buffer containing 5 mM Na2EDTA and 0.25% Triton X-100 (pH 7.6), and centrifugation at 2,300 g for 15 min at 4°C. Immunoprecipitates were further washed, twice, in 2 ml of 0.01 M phosphate buffer containing 15 mM NaCl (pH 7.2). When analysis by isoelectric focusing was attempted, immune complexes were precipitated by use of protein A as described below for neurophysins in incubation medium instead of a double antibody technique.

Neurophysins in incubation medium were similarly extracted using the antineurophysin sera. Before extraction, IgG of fetal calf serum included in the medium was removed by adding 30 mg of protein A-Sepharose CL-4B/ml (Pharmacia Fine Chemicals) and mixing for 2 h at 4°C in the presence of 10 μl/ml of the solution of 30 mg/ml of phenylmethylsulfonyl fluoride/30 mg/ml of iodoacetamide in ethanol. After centrifugation, 0.5–1 ml of the supernatant received the antisera (10 μl of the purified human antineurophysin serum or 100 μl of anti-NSN at 1:200 dilution) and incubation was performed by mixing for 24 h at 4°C. Immunoadsorptions were carried out by precipitating the immune complexes by the addition of 5 mg of protein A-Sepharose CL-4B followed by mixing for additional 2 h at 4°C and centrifugation at 2,300 g for 15 min. The immune complexes adsorbed to protein A-Sepharose were then washed as described above for the immunoprecipitates of cell extracts, and neurophysins were extracted with 0.5 ml of 1 N acetic acid and lyophilized.

**Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.** Neurophysins extracted from cells as well as incubation media were dissolved in 8 M urea/1.5% SDS/5% 2-mercaptoethanol/0.05 M sodium acetate/0.05 M boric acid (pH 8.5) by incubating at 70°C for 30 min and subjected to SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis (12.5% acrylamide/0.67% Na, N'-methylene bisacrylamide) was performed as originally described by Davies and Stark (15) with gel tubes of 10 × 0.5 cm and running buffer of 0.1 M borate/0.1 M sodium acetate/0.1% SDS (pH 8.5). Molecular weight markers used were the following: bovine serum albumin, egg albumin, α-subunit of E. coli DNA-polymerase, chymotrypsinogen, trypsin inhibitor, cytochrome c, 125I-labeled NSN, aprotinin, insulin chain B, and αα(1–39)ACTH. Each sample contained cytochrome c as an internal marker. After electrophoresis, gels were cut into 2-mm slices followed by elution into 1 ml of 0.1% SDS/0.5 M urea/5 mM sodium bicarbonate at 37°C for 16–20 h. 10 ml of scintillation fluid (Aquasol-2, New England Nuclear) was added and the radioactivity was counted in an Amersham-Searle Mark III liquid scintillation spectrometer (Amersham Corp., Arlington Heights, Ill.).

**Isoelectric focusing.** Isoelectric focusing in polyacrylamide gels was done according to the method described by O’Farrell (16). The sample buffer used with this gel system contained 8 M urea, 1% Triton X-100 and 2% ampholytes (nominal pH range 3.5–10.4; 1:1, vol/vol; LKB Producter AB, Bromma, Sweden). After focusing, 2-mm slices were cut and counted in 10 ml of toluene scintillator containing 3% Protosol (New England Nuclear).

**RESULTS**

When a tracer amount of 125I-labeled NSN was immunoprecipitated with the purified human antineurophysin serum and subjected to SDS-gel electrophoresis, a single peak of radioactivity was observed in the region of the molecular mass of 10,000 daltons with the recovery >90%. The presence of cell extracts containing 0.5–5 ng of immunoreactive neurophysin
depressed the binding of $^{125}$I-labeled NSN by <5%. Nonspecific binding was monitored by performing control immunoprecipitations with a sufficient amount of human neurophysin to exceed the binding capacity of the antiserum by 1,000-fold.

In Fig. 2 are shown the migration patterns on SDS-gel electrophoresis of the immunoprecipitates of cell extract and medium when cells were incubated with $^{35}$S-labeled cysteine for 12 h. By using the purified human antineurophysin serum for immunoprecipitation of cell extract, two major peaks of radioactivity were observed, molecular mass of which corresponded to 10,000 (10K) and 20,000 (20K), respectively (Fig. 2A). In addition, a small peak of radioactivity was found in the region of the molecular mass of 45,000–50,000 daltons. All forms of $^{35}$S]neurophysins were displaced from the immunoprecipitate by excess human neurophysin. When anti-NSN serum from NIH was used for immunoprecipitation of the same cell extract, the resolution profile of neurophysins on SDS-gel electrophoresis was qualitatively and quantitatively identical (Fig. 2B). Electrophoresis of neurophysins extracted from the incubation medium with the purified human antineurophysin serum, on the other hand, showed that neurophysins consist of two peaks, 10K and 20K species (Fig. 2C). Again, this pattern of neurophysins was not altered when anti-NSN serum was used for the extraction of neurophysins in the same medium (Fig. 2D). A large excess of human neurophysin in immunoprecipitation completely eliminated the label in two peaks in each instance. These results indicate that the purified human antineurophysin serum could be useful for the study of the kinetics of labeling of neurophysins in ectopic vasopressin-producing tumors. Because of the paucity of anti-NSN serum, this antiserum was used in the following experiments.

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**Figure 2.** Electrophoretic analyses, in SDS-polyacrylamide gels, of the immunoprecipitates of cell extract (A and B) and incubation medium (C and D). Cells were incubated with L-$^{35}$S]cysteine for 12 h. The purified human antineurophysin serum (A and C) or anti-NSN (NIH) (B and D) was used for immunoprecipitation. Open circles indicate the specific immunoprecipitate, while filled circles the human neurophysin-competed immunoprecipitate.
Comparison of the migration profiles of neurophysins in the cell extract and the incubation medium in Fig. 2 revealed that 20K peak was smaller than that of 10K in the former, while the opposite was observed in the latter. It has not been determined whether this quantitative difference could be attributable to selective secretion of 20K neurophysin, selective proteolysis, or interconversion after secretion. In view of the time-course of labeling of neurophysins described below, however, it may be more likely that synthesis and secretion of neurophysins did not reach an equilibrium at 12 h incubation.

Tumor cells were then incubated with \[^{35}S\]cysteine and \[^{3}H\]glucosamine hydrochloride for 12 h and the immunoprecipitate of the cell extract was analyzed by SDS-gel electrophoresis. As shown in Fig. 3A, a significant amount of tritium was incorporated into the 20K neurophysin region, while no \[^{3}H\]-labeled 10K neurophysin was demonstrated. When neurophysins immunologically extracted from the incubation medium were similarly processed, a peak of tritium was observed only in the region of 20K (Fig. 3B). The \[^{35}S]/\[^{3}H\] ratio in the 20K neurophysin region in the cell extract was 3.5, which was close to that in the incubation medium, 3.3.

In order to observe the kinetics of labeling of different forms of neurophysins in ectopic vasopressin-producing tumors, cells were incubated with \[^{35}S\]cysteine and harvested after increasing periods of time from 30 min to 6 h (Fig. 4). Incorporation of \[^{35}S\]cysteine into immunoprecipitable neurophysins in cell extracts proceeded for the entire 6-h incubation. After a 30-min incubation, most of the radioactivity resided in 20K peak with a small but a significant peak in the region of 10K. Increasing the exposure time to \[^{35}S\]cysteine resulted in an accumulation of label in 20K neurophysin, however, it apparently reached a plateau at 1-h incubation. In contrast, 10K neurophysin peak progressively increased in its height. At 6-h incubation, the radioactivity in 10K neurophysin species exceeded that in 20K peak. In addition to these two peaks, a small peak of radioactivity was observed in the region of larger molecular mass, which increased after longer periods of incubation (Fig. 4D). Because of a small amount of radioactivity in the peak, further characterization of this larger molecular mass peptide was not performed. In view of the result of pulse-chase experiment described below, however, the possibility that this peptide is a biosynthetic intermediate of neurophysins may be unlikely.

Fig. 5 depicts the result of pulse-chase experiment. Cells were exposed to a short pulse of \[^{35}S\]cysteine with minimal disturbances to the system and chased after varying periods of incubation with an excess of unlabeled cysteine. Immunoprecipitation of a sample incubated with labeled amino acid for 30 min showed that label had been incorporated mainly into 20K neurophysin. After a 30-min chase incubation, the amount of the radioactivity in 20K peak was decreased with an apparent initial half-life of 1 h, while the label in 10K neurophysin began to increase. At 2 h of chase, the radioactivity in 20K neurophysin was almost equal to that in 10K, which increased thereafter to exceed the former at 3 h of incubation.

After the 12-h incubation in \[^{35}S\]cysteine, the medium contained approximately the same amount of \[^{35}S\]-labeled immunoprecipitable materials as the cell extract (Fig. 2). However, the amount of labeled neurophysins secreted into media was smaller at shorter time of incubation used for pulse-labeling experiment: the label in immunoprecipitable neurophysins in incubation medium was negligible at 30 min and it corresponded to 37 and 46% of that in cell extracts at 3- and 5-h chase incubation, respectively. Analysis by SDS-gel electrophoresis of labeled neurophysins in incubation medium in pulse-chase experiment revealed that 20K neurophysin peak was greater than that of 10K neurophysin. The label in 20K peak

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Electrophoretic analyses, in SDS-polyacrylamide gels, of the immunoprecipitates from continuously labeled cells. Four identical dishes were incubated with L-[35S]cysteine and harvested after 30 (A), 60 (B), 120 (C) and 360 min (D) of incubation. Open circles indicate the specific immunoprecipitate, while filled circles the human neurophysin-competed immunoprecipitate.

![Graph](image)

**DISCUSSION**

The foregoing results clearly show that two species of immunoreactive neurophysins are biosynthesized in vitro in oat cell carcinoma cells with ectopic vasopressin production. That this was occasioned simply by the use of a heterologous antineurophysin antibody obtained from a patient with diabetes insipidus treated with vasopressin tannate is highly unlikely, since the antiserum was purified before use by affinity chromatography with human neurophysin-coupled agarose. The fact that the addition of human neurophysin in excess completely displaced both 10K and 20K neurophysin peaks from the immunoprecipitates may support this view. Moreover, the migration patterns on SDS-gel electrophoresis of 35S-labeled neurophysins were identical when anti-NSN serum (NIH) was used for immunoprecipitation. Both the purified human antineurophysin serum and anti-NSN serum (NIH) slightly crossreacted with ESN, however, the radioactive neurophysins thus immunoprecipitated corresponded to 83 and 76% of that in total immunoprecipitable neurophysins at 3- and 5-h chase, respectively. The results indicate that some of both forms of neurophysins were secreted into the medium during chase incubation.

When cells were harvested after the 30-min incubation in [35S]cysteine and the immunoprecipitate of cell extract was subjected to isoelectric focusing on polyacrylamide gel, one major labeled protein with pi of 5.7 was observed (Fig. 6). Several minor peaks were also found, the identities of which are at present unknown. The result suggests that 20K neurophysin is principally composed of the pi 5.7 protein.
Polyacrylamide isoelectric focusing of 20K neurophysin. Cells were harvested after the 30-min incubation in [35S]cysteine and the immunoprecipitate of cell extract was subjected to isoelectric focusing.

were judged to be vasopressin-related neurophysins in view of a minute quantity of ESN in tumor tissues. These results indicate that 20K neurophysin is produced, in addition to 10K neurophysin, in an ectopic vasopressin-producing tumor. This is consistent with our previous finding (5) that immunoreactive 20K neurophysin as well as 10K species exists in tumor tissues with ectopic vasopressin production when analyzed by gel filtration combined with NSN radioimmunoassay.

When tumor cells were incubated with labeled cysteine for 30 min, the major fraction of label appeared in 20K neurophysin. Analysis of the immunoprecipitate of cell extract by isoelectric focusing on polyacrylamide gels revealed that 20K neurophysin was principally composed of the protein with a pI of 5.7 (Fig. 6). After 1 h, the radioactivity in this neurophysin species reached a plateau. In contrast, the radioactivity in 10K neurophysin increased progressively and by 6 h, it exceeded that in 20K peak (Fig. 4). This pattern of labeling may support, though does not demonstrate, a
precursor-product relationship between 20K and 10K neurophysins.

To establish the precursor-product relationship more definitely, tumor cells were chased with a large excess of unlabeled cysteine after a brief period of labeling. Again, the majority of radioactivity was initially observed in 20K neurophysin. After this, the label in 20K neurophysin decreased, while that in 10K peak was increasing (Fig. 5). In studying the kinetics of labeling of the two forms of neurophysins, it may be necessary to take into account neurophysins secreted into culture medium. Since the amount of labeled neurophysins secreted over this time period was <50% of that in cell extract, the pattern of labeling could not be explained by the selective secretion of 20K neurophysin into the medium. One may argue that 10K neurophysin was not increased in proportion to a decrease in 20K species. The observation that both 10K and 20K neurophysins were detectable in the medium with preponderance of the latter during chase incubation may explain this apparent discrepancy. These results led us to the conclusion that 20K neurophysin is being converted to 10K neurophysin during incubation and that 20K neurophysin with a pI of 5.7 is proneurophysin in ectopic vasopressin-producing tumor cells.

Although it has been hypothesized by Sachs and his colleagues (17, 18) that vasopressin and its neurophysin are synthesized as a common precursor in the perikarya of the supraoptic and paraventricular nuclei in the hypothalamus, no direct evidence supporting this hypothesis has been forthcoming. Recently, high molecular weight forms of immunoreactive neurophysins (17,000 and 20,000 mol wt) have been identified in extracts of mouse hypothalamus (19). It was also shown that labeled cysteine injected adjacent to the supraoptic nucleus of the rat was rapidly incorporated into a 20,000-dalton protein that, in time, was converted to a 12,000-dalton protein (20, 21). Subsequently, this 20,000-dalton protein was found to crossreact with antineurophysin serum (22) and to liberate neurophysin and vasopressin-like peptide by trypsinization (23). Similarly, bovine hypothalamic mRNA has been shown to direct the synthesis of possible neurophysin precursors in cell-free system (24–26). These findings may be consonant with our conclusion alluded to above that 20K neurophysin demonstrated in this study is, in all probability, proneurophysin in men.

We have recently shown that a high molecular weight form of neurophysin with the molecular weight of 20K on gel filtration is consistently observed in oat cell carcinomas of the lung with ectopic vasopressin production (5). When this 20K neurophysin was subjected to limited proteolysis with trypsin, an equimolar amount of immunoreactive neurophysin and vasopressin was generated, elution profiles on gel filtration of which were not distinguishable from that of 10K neurophysin and synthetic arginine vasopressin, respectively. Coupled with this finding, 20K neurophysin that has been shown to be produced in vitro in an ectopic vasopressin-producing tumor tissue in the present study may be a common precursor to vasopressin and neurophysin in men, although further studies may be required for the confirmation of this hypothesis.

Of considerable interest is that glucosamine was incorporated into the 20K neurophysin region, but not into 10K species. The result agrees with our previous finding that a substantial portion of 20K neurophysin in ectopic vasopressin-producing tumors is glycosylated judging from its adsorption to concanavalin A (5). In favor of this view is the observation by Lauber et al. (19) that their tentatively identified neurophysin precursor in mouse hypothalamus was bound by concanavalin A-Sepharose. Very recently, Russell et al. (27) have also shown that a putative [35S]cysteine-labeled precursor for vasopressin-related neurophysin in rat hypothalamus is bound to concanavalin A. These studies may indicate that proneurophysin is glycosylated in several mammalian species including mouse, rat, and man. If in fact proneurophysin is a glycoprotein, then its molecular mass of 20,000 daltons may be overestimated, since glycopeptides in general are known to migrate abnormally in SDS-gel electrophoresis (28).

It may be argued that the biosynthetic sequence of vasopressin and its neurophysin in ectopic vasopressin-producing tumors may be different from that in human hypothalamus. To date, however, such a difference has not been demonstrated between most ectopic hormone-producing tumors and their normal glandular counterparts. A close resemblance in physicochemical properties of hormones and neurophysins in tumors and in human posterior pituitary gland (5) may support this thesis. In human hypothalamus, neurophysin may be synthesized by posttranslational processing from a glycosylated proneurophysin with an apparent molecular mass of 20,000 daltons and a pI of 5.7.

ACKNOWLEDGMENTS

We are grateful to Dr. M. Inui at the Tokyo Metropolitan Examiners' Office for human pituitary glands and to Sandoz Ltd. Basel, Switzerland for synthetic oxytocin.

The authors are indebted to the National Institute of Arthritis, Metabolism and Digestive Diseases and the National Pituitary Agency, U. S. Public Health Service for the generous gift of NSN and ESN radioimmunoassay kits.

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