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SHORT COMMUNICATION

Trans-synaptic Regulation of Ribonucleic Acid Biosynthesis in Rat Adrenal Medulla

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SUMMARY

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In rat adrenal medulla the increase in activity of afferent cholinergic axons elicited by exposure to 4° for 4 hr augmented the incorporation of [^{3}H]uridine into RNA. This change began 6 hr after stimulus application, reached a maximum at 8–10 hr, and was terminated by 16 hr. This increase preceded that of [^{3}H]leucine incorporation into tyrosine hydroxylase, but followed translocation of the catalytic units of adenosine cyclic 3′,5′-monophosphate-dependent histone kinase from the medullary cytosol to the nuclei. Adrenal denervation blocked histone kinase translocation and the stimulation of [^{3}H]uridine incorporation into RNA and of [^{3}H]leucine into tyrosine hydroxylase and other proteins elicited by cold exposure. RNA synthesis in nuclei isolated from the adrenal medullae of rats exposed to cold was greater than in medullae from normal rats; this increase was associated with stimulation of RNA synthesis dependent on RNA polymerase II, the enzyme responsible for the synthesis of RNA containing polyadenylic acid, which includes messenger RNA. The incorporation of [^{3}H]uridine into poly(A)-containing RNA *in vivo* was significantly higher in the intact medulla than in the contralateral denervated medulla 8 hr after exposure to cold. It can be inferred that the trans-synaptic stimulation of RNA synthesis enhances primarily the transcription of new messenger RNA, which seem to control the translation of tyrosine hydroxylase and other medullary proteins.

INTRODUCTION

In the adrenal medulla of rats, the activities of tyrosine hydroxylase and dopamine β-hydroxylase are enhanced by an increased impulse flow in the splanchnic nerve elicited by the application of stress (1–3) or the administration of certain drugs (4–7). Since a high proportion of adrenal medulla cells (chromaffin cells) are innervated exclusively by cholinergic axons, this tissue provides a suitable model for studying the trans-synaptic regulation of protein synthesis. Studies in this laboratory have indicated that an early activation of adenosine cyclic 3′,5′-monophosphate-dependent protein kinase in the medullary cytosol is a cAMP[1]-mediated obligatory event in the increase of tyrosine hydroxylase activity elicited trans-synaptically (7, 8). Moreover, the activated histone kinase has to be translocated to a particulate fraction, including nuclei, in order to increase the activity of tyrosine

[1] The abbreviations used are: cAMP, adenosine cyclic 3′,5′-monophosphate; TCA, trichloroacetic acid.

hydroxylase molecules (8–10). In adrenals, the trans-synaptically induced increment in tyrosine hydroxylase activity can be attributed entirely to an accumulation of tyrosine hydroxylase enzyme protein (11, 12), and occurs about 16 hr after the onset of a stimulation of nicotinic receptors lasting at least 2 hr. Using cold exposure as the stimulus and the technique of radioimmunoprecipitation with an antibody directed to purified tyrosine hydroxylase, we have demonstrated that the accumulation of tyrosine hydroxylase molecules is due solely to an increased rate of synthesis of this enzyme rather than to a reduced rate of its degradation (13, 14). The increase in enzyme synthesis begins about 8 hr after stimulus application and lasts for approximately 20 hr (13, 14). Furthermore, we have shown that in the adrenal medulla the synthesis of other proteins is increased during the trans-synaptic induction of tyrosine hydroxylase (14). In this study we report on the trans-synaptic regulation of poly(A)-containing RNA biosynthesis in adrenal medulla and we present data on the time duration and latency for the activation of medullary RNA synthesis.

To assess the participation of RNA synthesis in the trans-synaptic regulation of medullary protein synthesis, the incorporation of [^{3}H]5′-uridine (40 Ci/mmole, New England Nuclear) into medullary RNA was examined before and after stimulus application in rats with unilateral adrenal denervation. These rats (male, about 120 g, obtained from Zivic Miller Laboratories, Allison Park, Pa.) were exposed to 4° for 4 hr on the fifth day after denervation and were then brought back to room temperature. At the times indicated they received pulses of [^{3}H]uridine for 30 min. In rats kept at room temperature, at zero time, the amount of [^{3}H]uridine incorporated into cold TCA-insoluble material from adrenal medulla homogenates was similar in the intact and denervated tissues (Fig. 1). Since the ^{3}H present in the TCA precipitate could be reduced almost completely by alkaline hydrolysis of the tissue homogenate, it was inferred that the label was incorporated into RNA. Six hours after the application of stress, the incorporation of

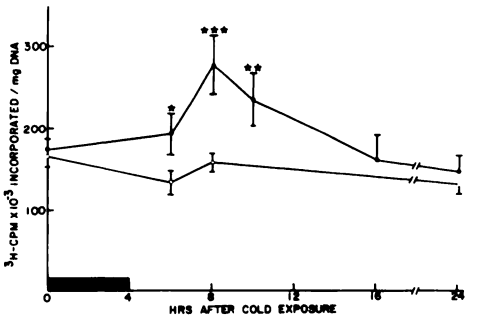

FIG. 1. *Effects of cold exposure on incorporation of [^{3}H]uridine into RNA in intact and denervated rat adrenal medullae*

Male rats with monolateral adrenal denervation were exposed to 4° for 4 hr (▬) on the fifth day after splanchnic nerve had been severed, as described previously (13, 14). At the times indicated, they received pulses of [^{3}H]uridine (10 mCi/kg intraperitoneally) for 30 min. Each intact and denervated medulla was dissected and homogenized in 0.5 ml of phosphate-buffered NaCl. (15). The incorporation of ^{3}H into RNA in intact (●) and denervated (○) medullae was assayed by precipitation with 5% cold TCA containing 0.05 M sodium pyrophosphate. The precipitates were collected by Millipore filtration and counted in a toluene-based scintillation fluid containing Triton X-100. DNA was determined by the fluorometric method, using ethidium bromide as the probe (15). Each value is the mean ± standard error of six experiments.

★ $0.02 < p < 0.05$ compared with the contralateral denervated medulla.

★★ $0.01 < p < 0.02$ compared with the intact medulla at zero time.

★★★ $0.002 < p < 0.01$ compared with the contralateral denervated medulla.

^{3}H into RNA in the intact medulla began to increase; however, the amount of isotope incorporated into the denervated medulla was virtually unchanged. At this time the increase in medullary cAMP content and the activation of soluble protein kinase are terminated (7, 8), but a greater amount of activated protein kinase (catalytic subunits) can be extracted from the 20,000 × *g* pellet of the homogenate (9) and from purified medullary nuclei (10). Between 8 and 10 hr the incorporation rate of ^{3}H into RNA in the intact medulla reached a peak about 70% above values in either the contralateral denervated medulla or the intact control (zero time). At 16 hr the isotope incorporation was identical in the

intact medullae of stressed and control rats. At this time the synthesis rate of adrenal tyrosine hydroxylase is maximally stimulated (13, 14) but the translocation of the catalytic unit of cAMP-dependent protein kinase begins to return to normal (9, 10); thus the trans-synaptic stimulation of nicotinic receptors in adrenal medulla augments the incorporation of [^{3}H]uridine into RNA after a time delay. This increase incorporation immediately follows the translocation of protein kinase but precedes the stimulation of tyrosine hydroxylase synthesis. It is of great interest that administration of actinomycin D up to 10–12 hr after the stress can block the trans-synaptic induction of adrenal tyrosine hydroxylase (6, 16). This time sequence suggests that the obligatory RNA transcription for tyrosine hydroxylase induction is complete by this time. Moreover, with unilateral adrenal denervation it was possible to document that the enhanced incorporation of [^{3}H]uridine into RNA, as well as the increased synthesis of tyrosine hydroxylase and other soluble proteins elicited by cold exposure, requires intact cholinergic innervation (14). Such a correlation strongly suggests that the trans-synaptic regulation of the synthesis of medullary proteins is mediated by a transcriptional rather than a translational control mechanism.

Since isolated nuclei offer the opportunity to estimate the participation of specific classes of RNA polymerases in the increase of RNA synthesis, avoiding the complications generated by changes in nucleotide pools, we isolated the nuclei from adrenal medullae pooled from 40 rats at zero time and 8 hr after exposure to 4°. RNA synthesis was measured in a medium that allows expression of the activity of RNA polymerase I as well as RNA polymerase II. The incorporation of [^{3}H]UTP into RNA in these isolated nuclei was measured in the absence and presence of various concentrations of α-amanitin, a specific inhibitor of RNA polymerase II (17). In the absence of α-amanitin, 30% more [^{3}H]UMP was incorporated into medullary nuclei prepared from rats exposed to cold than into nuclei from normal rats (Fig. 2). However, the difference in incorporation was progressively reduced by increasing amounts of α-amanitin. In fact, the rates of RNA synthesis resistant to α-amanitin were similar in nuclei isolated from the adrenal medullae of stressed and normal rats. In the bar graph in Fig. 2, the activity of RNA synthesis sensitive to α-amanitin inhibition was compared in nuclei from adrenal medullae of normal and stressed rats. Nuclei from stressed rats displayed 60% more RNA polymerase II activity than nuclei from normal rats. Thus, 8 hr after the initiation of cold exposure, nuclear RNA synthesis that is dependent on RNA polymerase II is enhanced while RNA polymerase I-dependent RNA synthesis appears to be unaffected. The increased synthesis of RNA in isolated medullary nuclei after cold expo-

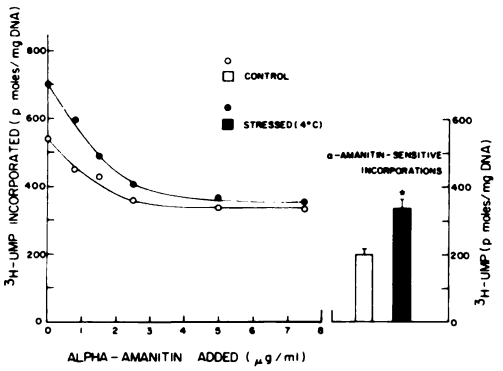

FIG. 2. *Effects of cold exposure on RNA synthesis in nuclei isolated from rat adrenal medullae*

Nuclei were isolated from medullae pooled from 40 rats kept at 25° and from 40 other rats 8 hr after exposure to cold. Nuclei were purified by washing through a 2 M sucrose solution according to the procedures reported (18). The recovery of DNA was about 55%, and the purity of the nuclei was routinely examined by light microscopy. The reaction conditions were those described by Marzluff *et al.* (18), with slight modifications. Each reaction mixture, in a volume of 200 μl, included nuclei containing 20–25 μg of DNA. Duplicate assay mixtures were incubated at 25° for 20 min. The counts obtained at zero time of incubation were regarded as background and subtracted. The bars represent the α-amanitin-sensitive synthesis of RNA, which was taken from the difference in incorporation without and with 5 μg/ml of α-amanitin. These values represent means ± standard errors of three experiments.

★ $p < 0.01$ compared with normal rats kept at 25°.

sure supports the concept that the maximal enhancement of [^{3}H]uridine incorporation into RNA observed *in vivo* reflects activation of the nuclear transcription machinery rather than a change in the compartmentation of UTP pools utilized for RNA synthesis.

In eukaryotic cells, almost all species of mRNA and some heterogeneous nuclear RNAs contain a poly(A) sequence at the 3′ ends of the molecules (19–21). Since RNA polymerase II catalyzes the synthesis of RNA containing poly(A) in the nucleoplasm (17, 22), it is important to examine whether the trans-synaptic stimulation of RNA transcription involves new synthesis of poly(A)-RNA. Thirty rats with unilateral adrenal denervation were injected with [^{3}H]uridine 8 hr after the beginning of a 4-hr cold stress and were killed 2 hr later. Intact and denervated medullae were pooled separately, and RNA was isolated by hot phenol extraction (23). The recovery of ^{3}H in RNA was about 60% for both denervated and intact medullae. The poly(A)-RNA was analyzed by oligo(dT)-cellulose chromatography (24). The [^{3}H]RNA bound to the column was significantly greater in the intact medulla than in the denervated medulla after exposure to cold (Fig. 3). In contrast, cold exposure increased the amount of unbound labeled RNA to a much smaller extent. In the intact medulla the ratio of bound [^{3}H]RNA [poly(A)-RNA] to unbound (bulk) RNA was about twice that in the contralateral denervated medulla. Thus an increase in poly(A)-RNA formation participates in the trans-synaptic increase of RNA synthesis elicited by cold exposure. This finding is in agreement with the increase in RNA polymerase II-dependent RNA synthesis *in vitro* found in medullary nuclei isolated from rats exposed to cold (Fig. 2).

The temporal correlation between the increase in mRNA synthesis and the activation and translocation of the activated protein kinase (8–10) is compatible with the view that stimulation of RNA synthesis is related to phosphorylation of some nuclear proteins. In nuclei, the rate of mRNA synthesis depends on the chromatin template activity and on the activity of

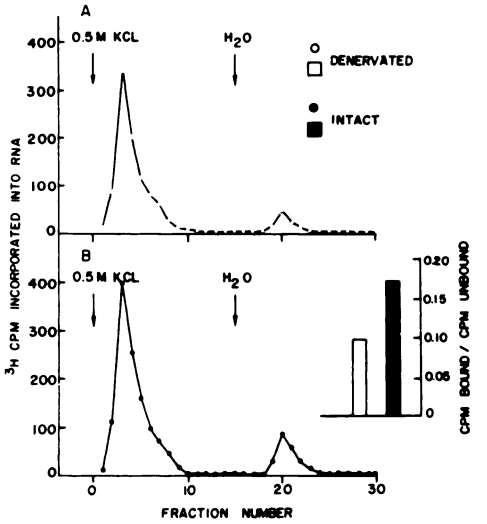

FIG. 3. *Incorporation of [^{3}H]uridine into RNA containing poly(A) in denervated (A) and intact (B) adrenal medullae of rats exposed to cold*

Thirty monolaterally denervated rats (about 120 g) were exposed to 4° for 4 hr on the sixth day after operation. Eight hours after the onset of stress, the rats received [^{3}H]uridine (1.2 mCi/rat intraperitoneally) and were decapitated 2 hr later. The intact and denervated medullae were pooled separately, and the labeled RNAs were extracted with hot phenol as described (23). After the final extraction, 25 A_{260} units of reticulocyte ribosomal RNA were added as a carrier and the RNA was precipitated with 2.5 volumes of ethanol (−20°). The purified RNA was chromatographed on a oligo(dT)-cellulose column (0.8 × 2 cm) in the presence of 0.5 M KCl, and the bound RNA [poly(A)-RNA] was eluted with Tris buffer (24). RNA in each fraction (0.5 ml) was precipitated with cold 5% TCA containing 0.05 M sodium pyrophosphate, using 100 μg of bovine serum albumin as a carrier. The ^{3}H in the TCA precipitates was collected on a Millipore filter and counted in a toluene-based scintillation fluid.

RNA polymerase II. In a variety of tissues, specific phosphorylated non-histone proteins may increase RNA synthesis in a system *in vitro* (25–27). On the other hand, it has been reported that the activity of ovarian RNA polymerase II can be stimulated by phosphorylation of the polymerase with protein kinase and cAMP (28). We have obtained preliminary evidence indicating that phosphorylation of adrenal medulla chromatin *in vitro* with cAMP and protein kinase enhances the template activity of chromatin, whereas medullary

RNA polymerase II incubated under similar phosphorylation conditions fails to increase its enzymatic activity. Thus we speculate tentatively that the trans-synaptic regulation of RNA synthesis is due to released template restriction of chromatin mediated by the phosphorylation of chromosomal proteins through the translocated protein kinase. Experiments to identify the natural nuclear substrate for the translocated protein kinase are in progress.

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