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ACTH and Brain RNA: Changes in Content and Labelling of RNA in Rat Brain Stem

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Abstract. The influence of synthetic $ACTH_{1-24}$ and $ACTH_{1-10}$ on rat brain RNA was studied. $ACTH_{1-24}$ (5 IU/100 g s.c.) treatment resulted in a small decrease in labelling of brain stem RNA (–12%), measured 30 min after the injection of [5-^{3}H] uridine (100 μCi; s.c.), without affecting the distribution of radioactivity of the acid-soluble precursor pool. Furthermore, a small and transitional reduction of total brain stem RNA was found. Similar treatment of adrenalectomized rats (36 h after surgery) resulted in a marked increase (+ 40%) in labelling of brain stem RNA, without affecting the distribution of radioactivity in the acid-soluble pool. In both intact and adrenalectomized rats, treatment with $ACTH_{1-24}$ did not affect RNA of cortex cerebrum and cerebellum.

$ACTH_{1-10}$, a fragment of ACTH without corticotropic activity *in vivo*, exhibited no effect on brain stem RNA in either intact or adrenalectomized rats, nor did corticosterone (1 mg/100 g) affect adrenalectomized rats.

Behavioral [DE WIED *et al.*, 1972], neuroendocrine [MOTTA *et al.*, 1965] and neurophysiological [STEINER, 1970; VAN DELFT and KITAY, 1972; ZIMMERMANN and KRIVOY, 1973] studies suggest that pituitary-adrenal hormones (ACTH and corticosteroids) may exert a direct effect on nervous tissue. However, there is little indication by what mechanism such an effect on the nerve cell would take place. These hormones seem to affect several cellular processes, i.e., catecholamine metabolism [VERSTEEG *et al.*, 1972; HÖKFELT and FUXE, 1972], glycerol phosphate dehydrogenase [DE VELLIS and INGLISH, 1968], RNA and protein metabolism [GISPEN and SCHOTMAN, 1973; REITH *et al.*, 1974], etc. The coherence of these various effects is poorly understood.

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JAKOUBEK *et al.* [1972] suggested that treatment with ACTH influenced the synthesis of rapidly labelled RNA in mouse nervous tissue. In previous work, we reported that after hypophysectomy, the labelling of both rapidly labelled and ribosomal RNA in rat brain had been reduced [GISPEN *et al.*, 1970], concomitant with a reduction in cellular RNA content. These effects were primarily found in medial diencephalon, mesencephalon, and medulla oblongata. Leucine incorporation into proteins and protein content appeared to be diminished in these subcortical brain regions, also [SCHOTMAN *et al.*, 1976]. Treatment of hypophysectomized rats with $ACTH_{1-10}$, a fragment of ACTH with full behavioral activity but lacking corticotropic activity [DE WIED, 1969] *in vivo*, did not alter the reduced incorporation of uridine into rapidly labelled brain stem RNA, but restored the poor incorporation of leucine into brain stem proteins [SCHOTMAN *et al.*, 1972; GISPEN and SCHOTMAN, 1973].

In the present study, the influence of 2 synthetic ACTH fragments ($ACTH_{1-24}$ and $ACTH_{1-10}$) on RNA metabolism in rat brain was studied. $ACTH_{1-24}$ has both the corticotropic and behavioral activity of the natural hormone, whereas $ACTH_{1-10}$ exerts the full behavioral activity without stimulating adrenal steroidogenesis *in vivo*.

Materials and Methods

Animals and surgery. Male albino rats of an inbred Wistar strain were used, weighing 110–120 g. In some experiments, rats were subjected to bilateral adrenalectomy, 36 h prior to use. Such rats were kept on saline instead of water.

Injected materials. Synthetic $ACTH_{1-24}$ (100 IU/mg) or synthetic $ACTH_{1-10}$ was dissolved in a drop of 0.01 M HCl and diluted with saline. The peptide solution was administered s.c. (0.2 ml): $ACTH_{1-24}$ in a dose of 50 μg/100 g b.w., an equivalent of 5 IU/100 g, and $ACTH_{1-10}$ in a dose of either 22.2 or 55.5 μg/100 g, being equimolar to 5 or 12.5 IU of $ACTH_{1-24}$. Corticosterone (Sigma) was injected s.c. in a dose of 1 mg/100 g b.w., freshly dissolved in 1 ml saline. To a batch of [5-^{3}H] uridine (1 or 5 Ci/mmol, Amersham), NaCl was added to final concentrations of 0.9%. From this solution of ^{3}H-uridine, 0.2 ml containing 100 μCi was injected s.c.

Experimental design. The treatment schedule was taken from JAKOUBEK *et al.* [1972]. At time zero (always 09.00 h a.m.), 1 group of rats was treated with $ACTH_{1-24}$, $ACTH_{1-10}$, or corticosterone, while another group received saline. 30 min later, all rats were injected with [5-^{3}H] uridine, and at time = 60 min, all animals were killed by decapitation. Subsequently, some ml of blood were collected and the brain was removed from the skull.

Tissue fractionation and biochemical procedure. A sample of peripheral blood was taken for determination of circulating corticosterone, using a protein-binding assay, according

to MURPHY [1967] and modified by VAN WIMERSMA GREIDANUS (unpublished). Another sample was used for analysis of the radioactivity present in the acid-soluble pool of blood plasma (see below).

The brain was dissected rapidly into cerebellum, brain stem (representing subcortical tissue caudal from the chiasma opticum) [GISPEN *et al.*, 1972], and remaining brain tissue, being mainly cortex cerebrum. These brain parts were weighed and washed twice for 10 min in 5 ml ice-cold buffer (0.01 M sodium phosphate, pH 7.2) to remove adhering fluid. All further treatment was performed at 0 °C, unless indicated otherwise. The tissue was homogenized in buffer using a loosely-fitting teflon pestle in a homogenizer, according to POTTER and ELVEHJEM [1936] (cerebellum and brain stem 3 ml each, cortex cerebrum in 6 ml). From the homogenate, 2 aliquots of 1.5 ml were taken. These samples were acidified with 0.5 M $HClO_4$ to a final concentration of 0.25 M $HClO_4$. After 20 min, the samples were centrifuged for 10 min at 10,000 *g*. The clear supernatants were saved for analysis of the acid-soluble pool (see below). The residues were washed 3 times with 0.25 M $HClO_4$. The walls of the tubes were dried with tissue paper, and the pellets were carefully resuspended in 1 ml of aqua destillata, using a glass rod. To these suspensions, 0.5 ml 0.9 M KOH was added, and the RNA present in these suspensions was hydrolyzed for 60 min at 37 °C. Hydrolysis was stopped in ice; 0.1 ml 9.2 M $HClO_4$ was added, and the samples were allowed to sit for 20 min. Acid-insoluble material was collected by centrifugation (10 min, 10,000 *g*). The acid-soluble fraction containing the hydrolyzed RNA was saved and pooled with 2 additional washings with 0.25 M $HClO_4$. The amount of RNA was determined by measurement at λ = 270 and 290 nm according to SPIRIN [1958].

Analysis of precursor pool. To the acid-soluble fraction (Sup A) of the homogenate, or blood plasma, about 1 μCi ^{14}C-uridine and 1 μCi ^{14}C-UMP was added, and nucleotides were isolated according to TSUBOI and PRICE [1959]. The nucleotides were adsorbed quantitatively to active charcoal. The charcoal suspension was spun for 10 min at 3,000 *g*, yielding a charcoal residue and a supernatant fluid (Sup B). The residue was washed twice, and the 2 supernatant fluids were added to Sup B. This combined fraction was taken to dryness under reduced air pressure at 30 °C. The dry residue was resuspended in water and dried again, yielding residue B (Res B). To estimate the content of tritiated water in Sup A, the amount of dis/min ^{3}H and ^{14}C were measured both in Sup A and Res B. The percentage of tritiated water was calculated by $\frac{\text{(Sup B — Res B)}}{\text{Sup A}} \times 100\%$ for ^{3}H, and correction of this value for the loss of radioactivity during the procedure was obtained by applying the same equation for dis/min ^{14}C.

Nucleotides were eluted from the washed charcoal and isolated as described before [Res C; GISPEN *et al.*, 1970]. Again the percentage of tritiated nucleotides $\frac{\text{Res C}}{\text{Sup A}} \times 100\%$ was corrected for eventual loss of radioactivity (loss in dis/min ^{14}C). Thus, the amounts of dis/min present in both nucleotides and water were determined independently. As can be seen in table II, the summations of the 2 percentages accounted for nearly all radioactivity in Sup A, indicating reliable, quantitative analysis. All measurements of radioactivity were carried out in a Mark II Nuclear Chicago Liquid Scintillation Counter, as described previously [GISPEN *et al.*, 1970]. In single isotope measurements, the efficiencies of ^{3}H and ^{14}C were about 43 and 55%, respectively. The counts per min were corrected for background activities and quenching, and computed to dis/min.

Results

$ACTH_{1-24}$ and Brain RNA in Intact Rats

One group of intact rats (N = 8) was treated with $ACTH_{1-24}$ (5 IU/100 g), another group with saline (N = 8). 30 min later, all rats received [5-^{3}H] uridine, and they were killed 30 min afterwards. Their brains were dissected, and both RNA content and incorporation of radioactive uridine into RNA were measured in brain stem, cerebellum and cortex cerebrum. The dis/min incorporated into RNA were expressed relative to the amount of radioactivity recovered from the whole brain part. This measure seems to be as close an estimation as possible of the process of incorporation from the endogenous pool of the precursor into RNA, and gives reproducible figures, as others have mentioned for the incorporation of amino acids into protein [RICHARDSON and ROSE, 1971].

One h after the administration of $ACTH_{1-24}$, a small but significant reduction in labelling of brain stem RNA could be detected (–12%, table I A). In the two other brain parts, no effect of treatment with $ACTH_{1-24}$ was observed, although there seemed to be a tendency to a reduced labelling of cerebellar RNA as well.

No effect on brain RNA content was found, 1 h after treatment with $ACTH_{1-24}$ in any of the 3 parts.

The relative amounts of radioactivity in water and nucleotides of brain stem were determined to see whether or not the observed reduction in labelling of RNA could be the result of changes in the precursor pool. As can be seen in table II, only about 20–25% of the radioactivity in the precursor pool could be attributed to nucleotides, whereas the bulk (about 75%) has been transferred to tritiated water. Treatment with $ACTH_{1-24}$ did not affect the distribution of radioactivity in the precursor pool.

Next, an eventual interference with precursor incorporation by changes in the uptake of labelled precursor from the circulation was considered. First, the distribution of radioactivity among free nucleotides and water in the precursor pool was analyzed in samples from peripheral blood, as was done for brain stem tissue (table II). The relative distribution appeared to resemble that found in brain stem tissue. Analysis of total amount of radioactivity in brain stem tissue (table III X) and blood plasma (table III Y) after the 30 min incorporation period revealed a small decrease of total amount of radioactivity recovered in brain stem and blood from rats treated with $ACTH_{1-24}$. However, the uptake of the precursor from the circulation by brain stem tissue (table III X/Y) was not affected by the treatment.

Table I. Effect of $ACTH_{1-24}$ on labelling and content of brain RNA, obtained from intact (A) and adrenalectomized (B) rats 1 h after injection of [5-^{3}H] uridine

Brain region	Treatment	dis/min in RNA, % of total radioactivity	Content of RNA, mg/g tissue
A			
Stem	Saline	0.80 ± 0.06[a] (8)	1.56 ± 0.01 (8)
	$ACTH_{1-24}$[b]	0.70 ± 0.04 (8)[c]	1.55 ± 0.01 (8)
Cerebellum	Saline	0.48 ± 0.04 (8)	2.47 ± 0.07 (6)
	$ACTH_{1-24}$	0.44 ± 0.05 (8)	2.49 ± 0.03 (6)
Cortex	Saline	0.70 ± 0.06 (8)	1.98 ± 0.03 (6)
	$ACTH_{1-24}$	0.73 ± 0.09 (8)	1.93 ± 0.03 (6)
B			
Stem	Saline	0.87 ± 0.09 (8)	1.53 ± 0.04 (8)
	$ACTH_{1-24}$	1.23 ± 0.09 (8)[d]	1.54 ± 0.04 (8)
Cerebellum	Saline	0.45 ± 0.04 (8)	2.53 ± 0.04 (6)
	$ACTH_{1-24}$	0.47 ± 0.03 (8)	2.35 ± 0.09 (8)
Cortex	Saline	0.74 ± 0.06 (7)	1.96 ± 0.04 (7)
	$ACTH_{1-24}$	0.74 ± 0.12 (8)	1.94 ± 0.06 (8)

[a] Mean ± SEM.
[b] $ACTH_{1-24}$ (5 IU/100 g).
[c] $p < 0.05$ (Student's *t*-test, two-tailed).
[d] $p < 0.01$ (Student's *t*-test, two-tailed).
( ) Number of rats.

Table II. Distribution of radioactivity in the acid-soluble pool from rat brain stem and blood plasma in intact (A) and adrenalectomized (B) rats

		Brain stem, % Recovered in		Blood plasma, % Recovered in	
		H_2O	Nucleotides	H_2O	Nucleotides
A	Saline (8)	77.6 ± 1.2[a]	22.1 ± 0.9	71.7 ± 3.7	25.7 ± 3.4
	$ACTH_{1-24}$[b] (8)	76.3 ± 1.0	21.7 ± 2.4	68.4 ± 3.5	20.8 ± 2.8
B	Saline (8)	76.7 ± 1.7	24.4 ± 2.0	—	—
	$ACTH_{1-24}$ (8)	78.0 ± 0.8	23.0 ± 0.8	—	—

[a] Mean ± SEM.
[b] $ACTH_{1-24}$ (5 IU/100 g).
( ) Number of rats.

Table III. Uptake of radioactivity by brain stem from blood in intact (A) and adrenalectomized (B) rats

		(X) dis/min × 10^{-3}/g, brain stem	(Y) dis/min × 10^{-3}/0.1 ml, plasma	(X)/(Y)
A	Saline (8)	824 ± 27[a]	143 ± 4	5.8 ± 0.3
	$ACTH_{1-24}$[b] (8)	749 ± 26[c]	135 ± 3	5.5 ± 0.3
B	Saline (8)	627 ± 33	141 ± 6	4.5 ± 0.3
	$ACTH_{1-24}$ (8)	618 ± 50	137 ± 9	4.5 ± 0.6

[a] Mean ± SEM.
[b] $ACTH_{1-24}$ (5 IU/100 g).
[c] $p < 0.05$ (Student's *t*-test, two-tailed).
( ) Number of rats.

Table IV. Brain stem RNA content 2.5 and 6 h after treatment with $ACTH_{1-24}$ in intact (A) and adrenalectomized (B) rats

		mg RNA/g tissue 2.5 h	6 h
A	Saline (6)	1.55 ± 0.01[a]	1.56 ± 0.05
	$ACTH_{1-24}$[b] (6)	1.47 ± 0.01[c]	1.51 ± 0.03
B	Saline (6)	1.55 ± 0.03	1.60 ± 0.03
	$ACTH_{1-24}$ (6)	1.60 ± 0.03	1.60 ± 0.02

[a] Mean ± SEM.
[b] $ACTH_{1-24}$ (5 IU/100 g).
[c] $p < 0.05$ (Student's *t*-test, two-tailed).
( ) Number of rats.

Table V. Effect of $ACTH_{1-24}$ on plasma corticosteroid levels in intact rats

	μg corticosterone/100 ml plasma 1 h	2.5 h	6 h
Saline (8)	15.0 ± 2.0[a]	5.7 ± 2.0	8.7 ± 3.5
$ACTH_{1-24}$[b] (8)	37.6 ± 1.5	31.2 ± 2.5	15.7 ± 3.0

[a] Mean ± SEM.
[b] $ACTH_{1-24}$ (5 IU/100 g).
( ) Number of rats.

More information on the effect of $ACTH_{1-24}$ on brain stem RNA content was obtained in a separate experiment in which RNA content was measured, 2.5 and 6 h after injection of either $ACTH_{1-24}$ (5 IU/100 g) or saline. In table IV A, a transitory and small reduction of brain stem RNA at time 2.5 h is shown. In both cortex and cerebellum, no influence of $ACTH_{1-24}$ was observed in this respect.

Table V contains the data on plasma corticosterone levels from rats used in the experiments. The mean values clearly indicate that in rats treated with $ACTH_{1-24}$, circulating corticosterone levels remain elevated throughout the 1st 6 h after administration of exogenous ACTH. This observation implies that at least 2 causative stimuli may exist: i.e., circulating exogenous $ACTH_{1-24}$, and endogenous corticosteroids. The experiments below try to assess the relative importance of these stimuli.

$ACTH_{1-24}$ and Brain RNA in Adrenalectomized Rats

To discriminate between a direct effect of $ACTH_{1-24}$ on RNA metabolism and an indirect influence via the release of corticosteroids, the previous experiments were repeated in adrenalectomized rats. The animals were adrenalectomized bilaterally 36 h prior to the first peptide or saline injection. It has been shown by others that after this interval, a negligible amount of corticosteroids circulates, whereas the level of endogenous ACTH is still low [DALLMAN *et al.*, 1972]. One group of adrenalectomized rats (N = 8) was treated with synthetic $ACTH_{1-24}$ (5 IU/100 g), another with saline (N = 8). 30 min later, all rats received [5-^{3}H] uridine, and after 30 min they were killed. Tissue fractionation and biochemical procedures were similar to those described for the experiments with intact rats. Treatment of adrenalectomized rats with $ACTH_{1-24}$ resulted in a marked increase in the labelling of brain stem RNA (table I B). No difference in this respect could be detected in the other brain areas studied.

The observed increase in labelling of brain stem RNA could not be attributed to differences in uptake of label or distribution of radioactivity in the precursor pool in brain stem tissue obtained from adrenalectomized rats treated with either placebo or peptide. Neither the ratio of total dis/min per g brain stem over total dis/min per 0.1 ml blood (table III B), nor the relative amounts of radioactivity in water and nucleotides of the precursor pool (table II B), were influenced by the treatment with $ACTH_{1-24}$. The apparent difference in table III between amount of radioactivity present in brain stem tissue of intact (A) and adrenalectomized rats (B) might have been caused by the difference in batch used in the separate experiments. The difference

was not consistent in a control experiment in which intact and adrenalectomized rats were compared (see also table VI). No changes in RNA content between the different groups of adrenalectomized rats were observed 1, 2.5 and 6 h after treatment with $ACTH_{1-24}$ (table I B, IV B).

$ACTH_{1-10}$ and Brain RNA in Intact and Adrenalectomized Rats

The N-terminal fragment of ACTH, $ACTH_{1-10}$, was used in order to obtain information on whether or not the neurochemical effect of treatment with $ACTH_{1-24}$ was exclusively related to the peptide sequence with full corticotropic properties. At first, intact rats were treated with synthetic $ACTH_{1-10}$ (22.2 μg/100 g; equimolar to 5 IU $ACTH_{1-24}$/100 g) or saline. 30 min later all rats received [5-^{3}H] uridine, and 30 min thereafter, all animals were killed. Both the incorporation of uridine into brain stem RNA and the plasma corticosterone levels were measured, as described for the previous experiments. As can be seen in table VI A, treatment of intact rats with $ACTH_{1-10}$ neither reduced the incorporation of uridine into brain stem RNA, nor elevated the level of circulating plasma corticosterone, both parameters being measured 1 h after the administration of $ACTH_{1-10}$. This experiment was repeated using adrenalectomized rats, as described previously. Two dose levels were used: i.e., a dose equimolar to 5 IU $ACTH_{1-24}$/100 g, and 2.5 times that dose. Contrary to $ACTH_{1-24}$, $ACTH_{1-10}$ did not increase the incorporation of uridine into brain stem RNA in these rats (table VI B). Therefore, it may be concluded that the ability of the corticotropic peptide $ACTH_{1-24}$ to produce modifications in brain stem RNA metabolism is not shared by the sequence $ACTH_{1-10}$.

Corticosteroids and Brain RNA in Adrenalectomized Rats

The apparent coincidence of corticotropic activity and effectiveness on brain stem RNA metabolism in the sequence $ACTH_{1-24}$ prompted us to study the effects of corticosteroids. Adrenalectomized rats were used to get maximal discrimination between treatment groups with respect to the plasma level of circulating corticosteroids. The same experimental conditions were used as in the previous experiments; however, in this study instead of ACTH, corticosterone (1 mg/100 g) was administered 30 min prior to the injection of [5-^{3}H] uridine. This treatment resulted in an increase of plasma corticosterone levels from 0.6 ± 0.3 in placebo treated adrenalectomized rats to 53.9 ± 2.7 μg/100 ml in corticosterone treated animals 60 min after the steroid administration. However, treatment with corticosterone affected neither the incorporation of uridine into brain RNA (table VII), nor the RNA content

Table VI. Effect of $ACTH_{1-10}$ on labelling of brain stem RNA and plasma corticosterone levels in intact (A) and adrenalectomized (B) rats

		dis/min in RNA, % of total radioactivity	Plasma corticosterone, μg/100 ml plasma
A	Saline (8)	0.85 ± 0.03[a]	16.0 ± 5.2
	$ACTH_{1-10}$[b] (8)	0.86 ± 0.04	20.7 ± 4.2
B	Saline (8)	0.72 ± 0.04	—
	$ACTH_{1-10}$[b] (8)	0.79 ± 0.06	—
	Saline (8)	0.89 ± 0.06	—
	$ACTH_{1-10}$[c] (8)	0.83 ± 0.04	—

[a] Mean ± SEM.
[b] ~ 5 IU $ACTH_{1-24}$/100 g.
[c] ~ 12.5 IU $ACTH_{1-24}$/100 g.
( ) Number of rats.

Table VII. Effect of corticosterone on labelling and content of brain RNA obtained from adrenalectomized rats 1 h after injection of [5-^{3}H] uridine

Brain region	Treatment	dis/min in RNA, % of total radioactivity	Content of RNA, mg/g tissue
Stem	Saline	0.83 ± 0.05 (8)[a]	1.52 ± 0.03 (8)
	Corticosterone[b]	0.88 ± 0.05 (8)	1.55 ± 0.03 (8)
Cerebellum	Saline	0.47 ± 0.05 (8)	2.51 ± 0.08 (8)
	Corticosterone	0.59 ± 0.08 (8)	2.44 ± 0.03 (8)
Cortex	Saline	0.72 ± 0.05 (8)	1.95 ± 0.08 (8)
	Corticosterone	0.74 ± 0.06 (8)	1.97 ± 0.10 (8)

[a] Mean ± SEM.
[b] Corticosterone (1 mg/100 g).
( ) Number of rats.

Table VIII. Uptake of radioactivity by brain stem from blood in adrenalectomized rats

	(X) dis/min × 10^{-3}/g, brain stem	(Y) dis/min × 10^{-3}/0.1 ml, plasma	(X)/(Y)
Saline (8)	743 ± 42[a]	174 ± 19	4.5 ± 0.3
Corticosterone[b] (8)	742 ± 61	137 ± 10	5.0 ± 0.3

[a] Mean SEM.
[b] Corticosterone (1 mg/100 g).
( ) Number of rats.

(table VII), nor the uptake of radioactivity from the blood stream into the brain stem (table VIII) under these conditions.

Discussion

In intact rats, a single, high dose of synthetic $ACTH_{1-24}$ resulted in a rather small, but significant inhibition of the incorporation of uridine into brain stem RNA 1 h after the treatment (table I, –12%). An incorporation period of 30 min was used, since it will almost exclusively label the rapidly labelled RNA [GISPEN *et al.*, 1970; JAKOUBEK *et al.*, 1972].

30 min after s.c. injection of [5-^{3}H] uridine, only about 22% of the radioactivity in the acid-soluble pool remained in nucleotides, and about 77% consisted of tritiated water (table II). This rapid loss of ^{3}H-label from the precursor to metabolites after peripheral injection has been reported for both nucleotides and amino acids [JAKOUBEK *et al.*, 1972; BANKER and COTMAN, 1971; SCHOTMAN *et al.*, 1974]. However, in spite of this effective loss of radioactive precursor after s.c. administration, this phenomenon could not account for the change in labelling of brain stem RNA, since treatment with $ACTH_{1-24}$ did not affect the distribution of radioactivity in the precursor pool (table II).

No differences were observed in either the uptake of total radioactivity from the circulation (table III X/Y), or the composition of the labelled compounds of the precursor pool in blood plasma (table II), as a result of treatment with $ACTH_{1-24}$. The radioactivity in the precursor pool of blood plasma was similar to that found in the pool of brain tissue (table II), indicating that none of the labelled components of the acid-soluble pool were taken up from the circulation by brain stem tissue preferentially. The lower amount of radioactivity present in blood and brain stem of $ACTH_{1-24}$-treated rats therefore seems rather the result of a peripheral factor than of an effect of the peptide on brain precursor metabolism.

Furthermore, a small decrease in brain RNA content occurred, 2.5 h after injection of $ACTH_{1-24}$, implying that ribosomal RNA is also involved. The data on both uridine incorporation and RNA content strongly suggest that brain RNA metabolism is affected by treatment with $ACTH_{1-24}$. These changes corroborate the findings of JAKOUBEK *et al.* [1972], with respect to magnitude and transitory nature, and may be related to the transient alteration in brain metabolism observed in young rats treated with synthetic ACTH [PALO and SAVOLAINEN, 1974].

It appeared that the effect was restricted to brain stem tissue, which included thalamus, caudal basal ganglia, mesencephalon, and medulla oblongata. These data support earlier observations that the metabolism of macromolecules in this particular brain region is sensitive to influences of the pituitary-adrenal hormones [GISPEN *et al.*, 1972; GISPEN and SCHOTMAN, 1973]. In adrenalectomized rats treated with ACTH, the incorporation of uridine into rapidly labelled RNA was increased by 22%. Thus, in animals with low corticosteroid and ACTH levels [DALLMAN *et al.*, 1972] an acute dose of $ACTH_{1-24}$ results in a stimulation of uridine incorporation, whereas under similar experimental conditions in animals with an intact pituitary-adrenal system, a decrease was found. One explanation would be that corticosteroids inhibit the uridine incorporation into brain stem RNA. Under certain experimental conditions, inhibitory influences of steroid hormones on brain macromolecule metabolism have been reported [DE KLOET and MCEWEN, 1976]. However, we were unable to detect such changes, as a result of treatment of adrenalectomized rats with corticosterone, according to the same experimental schedule as was used in the experiments with ACTH: i.e., an elevation of the low plasma corticosterone level to about 50 μg/100 ml was insufficient to alter uridine incorporation into brain stem RNA. However, it may be that the time differences between brain-steroid and brain-peptide interaction [GISPEN *et al.*, 1975] make it difficult to draw firm conclusions from these data. Therefore, it may be that, despite the negative results of steroid substitution in adrenalectomized rats, the absence or presence of a permissive influence of corticosterone is responsible for the opposite effects which $ACTH_{1-24}$ brings about in intact and adrenalectomized rats. $ACTH_{1-10}$, lacking the adrenocorticotropic activity, produced neither a decrease nor an increase in the labelling of brain RNA in either intact or adrenalectomized rats. READING and DEWAR [1971] reported a similar lack of effect of $ACTH_{4-10}$ on brain RNA in intact rats. In hypophysectomized rats treated with $ACTH_{1-10}$ for 12 days, no change of uridine incorporation was found, either. The ineffectiveness of treatment of adrenalectomized rats with $ACTH_{1-10}$, in comparison to $ACTH_{1-24}$, suggests that the peptide-sensitive structure in the brain stem discriminates between $ACTH_{1-10}$ and $ACTH_{1-24}$. It would appear that $ACTH_{1-24}$ affects brain macromolecule metabolism at the transcriptional level [see also JAKOUBEK *et al.*, 1972], whereas $ACTH_{1-10}$ and $ACTH_{4-10}$ express their effect at the translational level [SCHOTMAN *et al.*, 1976; SCHOTMAN and GISPEN, 1974; READING and DEWAR, 1971; RUDMAN *et al.*, 1974].

Although in studies on avoidance behavior, it is apparent that both

peptides are able to affect central nervous functioning in the same direction via an extraadrenal mechanism [DE WIED, 1969], in neonatal studies on somatic and behavioral development of the rat, similar differences between various ACTH analogues became apparent [VAN DER HELM-HYLKEMA, 1973]. It was found that $ACTH_{1-24}$ and $ACTH_{1-16}$ (the latter peptide showing hardly any corticotropic activity) accelerated eye opening in rats, whereas $ACTH_{1-10}$ appeared to be ineffective. It was argued that this extraadrenal effect of synthetic ACTH fragments would be mediated through an action on central nervous structures [VAN DER HELM-HYLKEMA, 1973]. The present study presents further evidence for possible differences in action of various ACTH fragments on brain structures, warranting caution for any generalization on neurochemical events brought about by ACTH-like peptides.

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