Advances in Biochemical Psychopharmacology, Vol. 6
Raven Press, New York © 1972

Hypophysectomy and Rat Brain Metabolism: Effects of Synthetic ACTH Analogs

D. H. G. Versteeg, W. H. Gispen, P. Schotman, A. Witter, and D. de Wied

Rudolf Magnus Institute for Pharmacology and Department of Physiological Chemistry, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

INTRODUCTION

For several years our group has been interested in the role of the hormones of the pituitary-adrenal system in avoidance conditioning in the rat. In particular, the performance of the hypophysectomized rat has been investigated in some detail (for a review, see de Wied, 1969). It was found that hypophysectomy markedly impaired the acquisition of a conditioned avoidance response in a shuttle box.

Treatment of hypophysectomized rats with ACTH restored the deficient performance toward almost normal levels (de Wied, 1964). However, experiments with MSH, which hardly affects adrenocortical activity, and with the synthetic ACTH analogs $ACTH_{1-10}$ and $ACTH_{4-10}$, which lack endocrine and metabolic effects, also showed a similar facilitation of avoidance conditioning in hypophysectomized rats (de Wied, 1969). Treatment of control rats with the same peptides delayed the extinction of a conditioned avoidance response (de Wied, 1966; de Wied, Bohus, and Greven, 1968; van Wimersma Greidanus and de Wied, 1971). Moreover, $ACTH_{1-10}$ — 7-D-phe facilitated extinction under the same experimental conditions in normal rats, and failed to facilitate acquisition of a conditioned avoidance response in hypophysectomized rats (Bohus and de Wied, 1966; de Wied, 1969). These experiments were interpreted as indicating that the pituitary contains neuropeptides which may affect formation of new behavior patterns.

On the basis of these data, a program was started to investigate the effects of hypophysectomy on brain metabolism and the possible effects of synthetic ACTH analogs on central nervous system metabolism in hypophysectomized rats.

HYPOPHYSECTOMY AND BRAIN METABOLISM

Hypophysectomy leads to dramatic disorders in metabolic processes in endocrine organs and other peripheral organs and tissues. As a consequence of the disturbance of hormonal regulating mechanisms, atrophy occurs in endocrine organs which are normally under the control of pituitary hormones, and metabolic changes can be observed in organs such as liver and muscle. The extensive literature dealing with the effects of hypophysectomy on metabolic parameters in peripheral organs and tissues will not be reviewed here.

Relatively little is known about the effects of hypophysectomy on the metabolism of the central nervous system. Reiss (1961) found little change in oxygen consumption of the cortex following hypophysectomy, while anaerobic glycolysis increased by more than 100%. Libertun, Moguilevsky, Schiaffini, and Foglia (1969) and Moguilevsky, Libertun, and Foglia (1970) observed a significant increase in oxygen uptake in certain parts of the hypothalamus of hypophysectomized rats. Although the effect of hypophysectomy on macromolecular metabolism has not been extensively studied, some evidence has been obtained for alterations in such metabolism. Decreases have been observed in both phenylalanine incorporation in a brain cell-free system and in poly-U stimulated amino acid incorporation in a system prepared from brain tissue following hypophysectomy (Dunn and Korner, 1966). Takahashi, Penn, Lajtha, and Reiss (1970) reported that after hypophysectomy the incorporation of isotopically labeled phenylalanine into brain protein was diminished. Hypophysectomy was found to lead to a significant decrease in RNA/DNA ratio in the brainstem (DeVellis and Inglish, 1968). Significant decreases in weight of the cortex and in content of DNA, RNA, protein, and water were found in the brains of young rats 3 weeks after hypophysectomy (Cheek and Graystone, 1969).

Data concerning monoamine levels and turnover in brain or brain regions of hypophysectomized rats are controversial. DeMaio (1959) found an increase in the serotonin level in medulla oblongata and brainstem after hypophysectomy, but Yeh, Solomon, and Chow (1959) could not confirm these findings. Resnick and Gray (1961) were also unable to detect any changes in brain levels of serotonin in hypophysectomized rats. How-

ever, Hyyppä and Valavaara (1970) reported an increase in the noradrenaline and serotonin content of anterior and posterior hypothalamus, but not of the cortex, 3 months after hypophysectomy. An increase in noradrenaline content of hypothalamic regions of the brain of hypophysectomized rats was also described by Shchedrina (1970). These findings suggest an effect of removal of the pituitary on monoamine levels in circumscribed brain areas, while levels in other regions of the brain seem unaffected. Few data are available concerning monoamine turnover. Landsberg and Axelrod (1968) described an unchanged noradrenaline turnover in the brain of rats 62 days after hypophysectomy; Fuxe, Corrodi, Hökfelt, and Jonsson (1970) mentioned a reduction in noradrenaline turnover in central neurons of hypophysectomized rats.

In view of these data, we felt it would be desirable to extend these observations concerning macromolecular and monoamine metabolism in the brains of hypophysectomized rats. Since the data should have a direct bearing on the influence of hypophysectomy on conditioned avoidance behavior, the same time schedule was used as in the previous behavioral experiments.

HYPOPHYSECTOMY AND MACROMULECULAR AND MONOAMINE METABOLISM IN THE BRAIN

In all experiments, except those to study monoamine metabolism, male rats were used. Hypophysectomy was performed via the transauricular route under light ether anesthesia on rats weighing approximately 120 g. Two or 3 weeks after hypophysectomy or sham operation, the rats were sacrificed by decapitation. Brains were dissected out immediately after the decapitation and processed as described below. Decrease in body weight, adrenal atrophy, and macroscopical inspection of the sella turcica were used as parameters for the completeness of the removal of the pituitary.

Total RNA Content

To investigate the effect of hypophysectomy on RNA metabolism in the brain, first total RNA content was determined in different brain areas. The brains were dissected into brainstem, cortex, and cerebellum (see Gispen, Schotman, and de Kloet, *in press*). RNA content was determined by the method of Munro and Fleck (1966); DNA content was measured

TABLE 1. *Gross localization of the effect of hypophysectomy on RNA content in rat brain*

Location	RNA (mg/g fresh tissue)	DNA (mg/g fresh tissue)	Ratio RNA/DNA
Intact			
Cortex (5)	1.77 ± 0.04	1.03 ± 0.06	1.74 ± 0.07
Cerebellum (4)	2.07 ± 0.08	4.91 ± 0.29	0.43 ± 0.01
Brainstem (4)	1.46 ± 0.05	0.99 ± 0.04	1.47 ± 0.04[a]
Hypophysectomized			
Cortex (6)	1.55 ± 0.02	1.04 ± 0.04	1.51 ± 0.07
Cerebellum (5)	1.90 ± 0.04	4.83 ± 0.23	0.40 ± 0.01
Brainstem (5)	1.35 ± 0.03	1.13 ± 0.03	1.19 ± 0.04[a]

No. of animals in parentheses. Values are mean ± SEM.
[a] $p < 0.025$ (t-test).

following the method of Burton (1956), using calf thymus DNA as a standard.

From Table 1 it can be seen that there is no significant difference in the RNA/DNA ratio, which represents a measure of RNA content per cell, between sham-operated and intact control rats weighing 120 g on the day of analysis. A lower RNA/DNA ratio was found in the brainstem of hypophysectomized rats, but not in the cortex and cerebellum. These data are in good agreement with the findings of De Vellis and Inglish (1968), who suggested that the effect of hypophysectomy on brain RNA was located mainly in brainstem areas.

Further studies revealed that in the brainstem, where the largest decrease was found in RNA/DNA ratio, the diencephalon, the mesencephalon, and the medulla oblongata all showed a decrease in RNA content following hypophysectomy (Gispen et al., *in press*). For these reasons, the brainstem was chosen for further study.

Subcellular Localization: Polysomes

With the same techniques of analysis, an attempt was made to localize the effect of hypophysectomy on RNA content at a subcellular level. Brainstems of hypophysectomized and control rats were homogenized and fractionated as described previously (Gispen, de Wied, Schotman, and Jansz, 1970). RNA content of homogenate, cell nuclei, mitochondria plus synaptosomes, microsomes, and the postmicrosomal supernatant was determined. As can be seen from Table 2, the only significant difference in RNA content between fractions from hypophysectomized and control rats

TABLE 2. *Subcellular localization of the effect of hypophysectomy on brainstem RNA*

Location	Brainstem RNA (mg/g fresh tissue)			
	Intact rats	Hypophysectomized rats	%[a]	p[b]
Homogenate	1.727 ± 0.002 (9)	1.425 ± 0.002 (11)	−17.5	<0.001
Purified nuclei	0.020 ± 0.002 (4)	0.018 ± 0.002 (4)	−10.0	n.s.
Crude mitochondria	0.156 ± 0.011 (7)	0.163 ± 0.010 (11)	+ 4.5	n.s.
Microsomes	0.352 ± 0.018 (7)	0.287 ± 0.009 (11)	−18.5	<0.01
Post-microsomal supernatant	0.379 ± 0.030 (7)	0.341 ± 0.014 (6)	−10.0	n.s.

Values are mean ± SEM. No. of animals in parentheses.

[a] Proportional difference between values obtained from intact and hypophysectomized rats setting the value of intact rats at 100%.

[b] *t*-test.

was found in the fractions containing the microsomes. This result indicates that hypophysectomy might alter macromolecule metabolism by a deficiency on the ribosomal or polysomal level, as suggested by Korner (1969) for liver tissue.

The main RNA components of the microsomal fraction are the ribosomal RNA's, and further *m*-RNA and *t*-RNA's in minor quantities. Thus, a decrease in the microsomal RNA content suggests a decrease in ribosomes and polysomal aggregates. To test this hypothesis, polysomes were isolated using a technique described by Adair, Wilson, and Glassman (1968). The polysomal fraction was separated into monosomes, disomes, trisomes, and others by centrifugation through linear sucrose gradients (Gispen et al., 1970). The amounts of monosomes, disomes, trisomes, and large polysomes were determined by computing the areas under the peaks, obtained by a continuous recording of the optical density of the gradients at 260 nm. In Fig. 1, the mean values of intact and hypophysectomized rats are compared. It can be seen that hypophysectomy affects mainly the content of polysomes, especially of large polysomes (≥ trisomes), implying a reduced number of structures active in protein synthesis.

Uridine Incorporation into RNA

We next investigated whether the reduction in polysome content following hypophysectomy was due to a decreased RNA synthesis or to a decreased stability of the polysomal aggregates. For liver tissue, a decreased

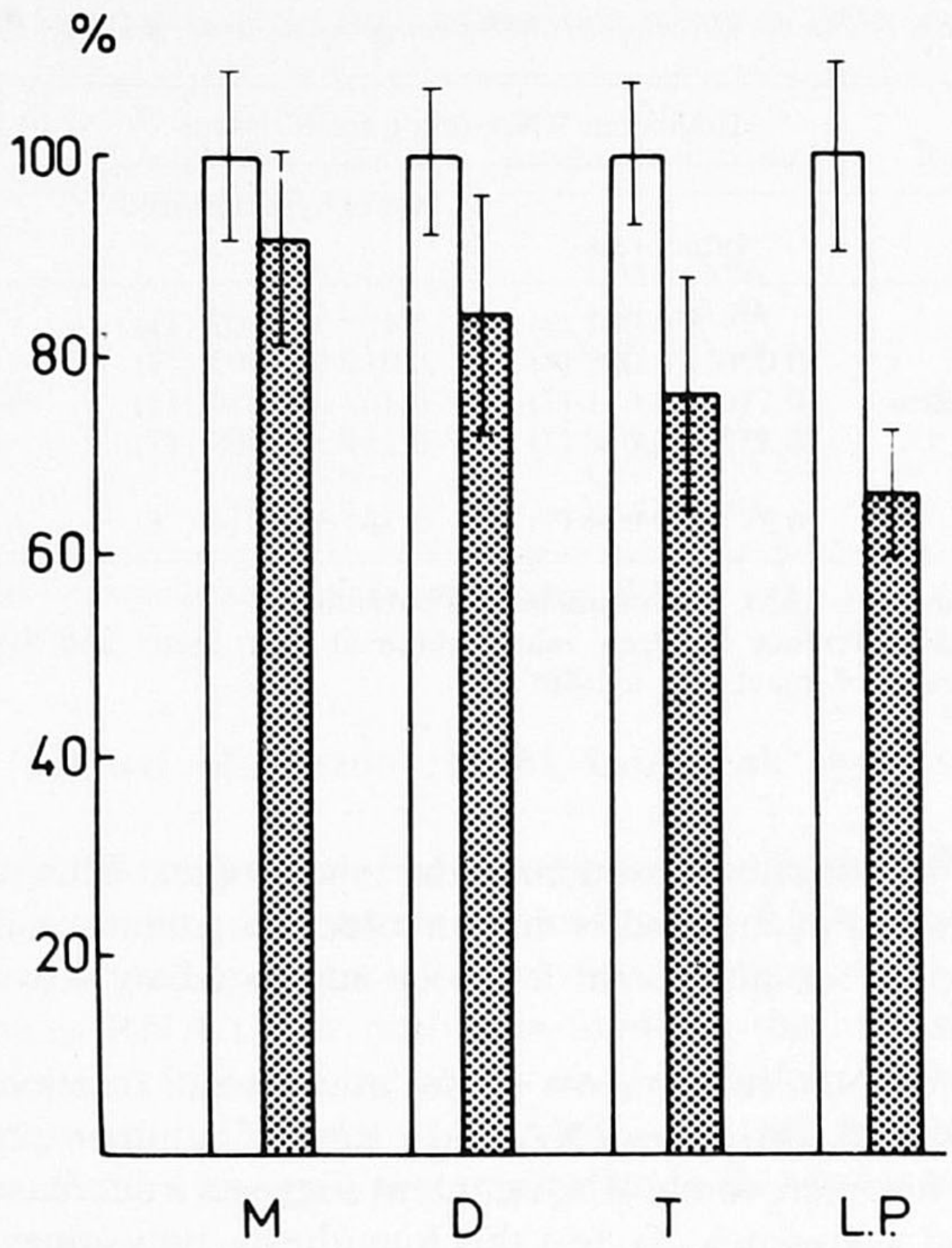

FIG. 1. Effect of hypophysectomy on content of monosomes (M), disomes (D), trisomes (T), and large polysomes (LP) in the rat brainstem. Values obtained for fractions from brainstems of hypophysectomized rats are expressed as percent of values for fractions from brainstems of intact rats. *Open columns:* intact rats; mean ± SEM; n = 15. *Stippled columns:* hypophysectomized rats; mean ± SEM; n = 11. For hypophysectomized trisomes, $p < 0.005$. For hypophysectomized large polysomes, $0.01 < p < 0.025$. (p values determined by t-test).

RNA synthesis, an increased RNAse activity, and a ribosomal deficit have been suggested to explain the changes in macromolecule metabolism in the hypophysectomized rat (Korner, 1970; Cardell, 1967; Brewer, Foster, and Sells, 1969; Foster and Sells, 1969). However, in brain tissue no ribosome deficit has been found (Dunn and Korner, 1966). Therefore, the turnover of RNA in the brainstem of hypophysectomized rats was studied. This was carried out by using radioactive labeled uridine as a precursor for RNA.

In order to study the *in vivo* incorporation of radioactive labeled uridine into different cell fractions, a double labeling method was used, in which (5-^{3}H)- or (2-^{14}C)-uridine was administered by injection into the dien-

cephalon (Valzelli, 1964) either alternatively to control and hypophysectomized rats or to two control rats. The incorporation of the ^{3}H- and ^{14}C-label into several RNA fractions after 70 and 180 min of incorporation was measured and corrected for the relative ^{3}H- and ^{14}C-radioactivity of the precursor pool as described before (Gispen et al., 1970). Using two

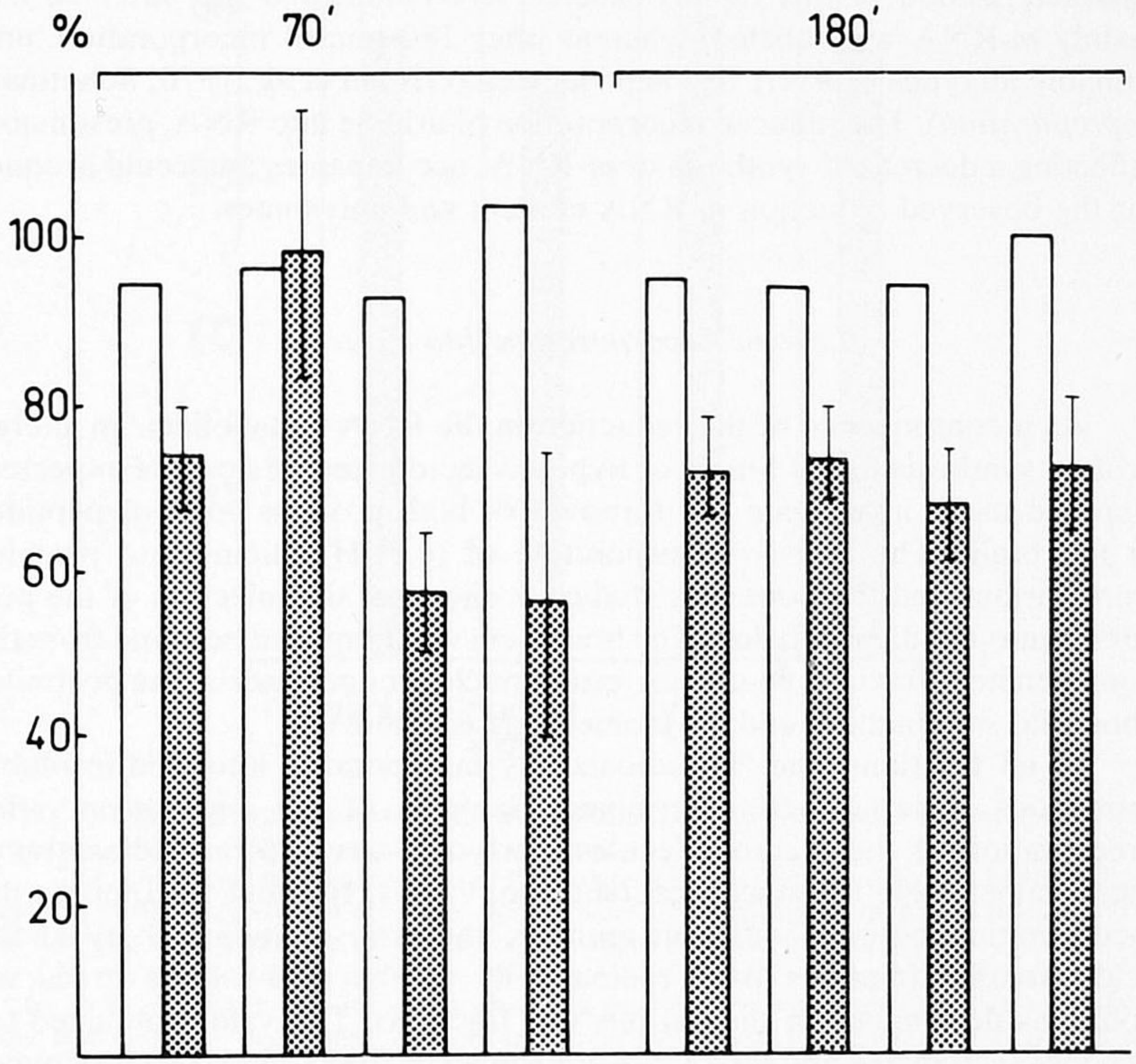

FIG. 2. Effect of hypophysectomy on (5-^{3}H)- and (2-^{14}C)-uridine incorporation into total, nuclear, cytoplasmic, and polysomal RNA in the rat brainstem.

The ratio of ^{3}H- and ^{14}C-label or ^{14}C- and ^{3}H-label in the RNA fractions was corrected for the ratio in the precursor pool. In the figure, the corrected ratios obtained for pairs consisting of one hypophysectomized and one intact rat are expressed as percent of the ratios obtained for pairs of two intact rats, which did not differ from unity.

Open columns: ratios of incorporation of label into RNA fractions from the brainstems of two intact rats. *Stippled columns:* ratios of incorporation of label into RNA fractions from the brainstems of a pair consisting of one hypophysectomized and one intact rat. Mean ± SEM; n = 3.

intact rats, one receiving ^{3}H- and the other ^{14}C-uridine, the corrected isotope ratio of the cell fractions differed by less than 7% from the theoretical value of one. However, using pairs of one hypophysectomized and one control rat, the isotope ratio was 25 to 40% lower, indicating a decreased incorporation of uridine into RNA in the brainstem of hypophysectomized rats (Fig. 2). The decrease was not limited to any particular cell fraction. Characterization of this rapidly labeled RNA indicated that after 70 min mainly *m*-RNA was labeled, whereas after 180 min of incorporation, presumably all types of RNA had been labeled (Gispen et al., 1970; Schotman, *in preparation*). The reduced incorporation of uridine into RNA, presumably reflecting a decreased synthesis of *m*-RNA, accompanies and could account for the observed reduction in RNA content and polysomes.

Leucine Incorporation into Proteins

As a consequence of the reduction in the RNA metabolism, an altered protein synthesis in the brains of hypophysectomized rats can be expected. This led us to investigate the turnover of both proteins and polypeptides in the brain. The *in vivo* incorporation of (4,5-^{3}H)-leucine into proteins from various cell fractions was studied 5 min after the injection of the precursor into the diencephalon. The brainstem was homogenized, and from the homogenate a fraction containing crude nuclei, mitochondria, the postmitochondrial supernatant, and polysomes was isolated.

In all fractions, the ^{3}H-radioactivity incorporated into acid-insoluble proteinous material was determined. Samples of the supernatant after precipitation of the macromolecules were also assayed for radioactivity; these values were taken as precursor pool values. In order to compare the incorporation between different animals, the ratio of radioactivity of the acid-insoluble fractions over radioactivity of the acid-soluble precursor pool was determined in the various cell fractions. The values obtained for hypophysectomized and intact rats are shown in Fig. 3. As a result of hypophysectomy, a decrease in leucine incorporation was observed, which was about equal in all cell fractions. Only a minor part of the labeled insoluble material appeared to consist of RNAse-sensitive material (probably labeled aminoacyl-*t*-RNA), whereas a large part could be degraded by incubation with pronase. The latter result indicates that the rapidly labeled material is of a protein nature (Schotman, *in preparation*). A short incorporation time was used, since it was found in pilot studies that 5 min after injection of ^{3}H-leucine into the brainstem, the radioactivity in the acid soluble precursor pool was at a maximum.

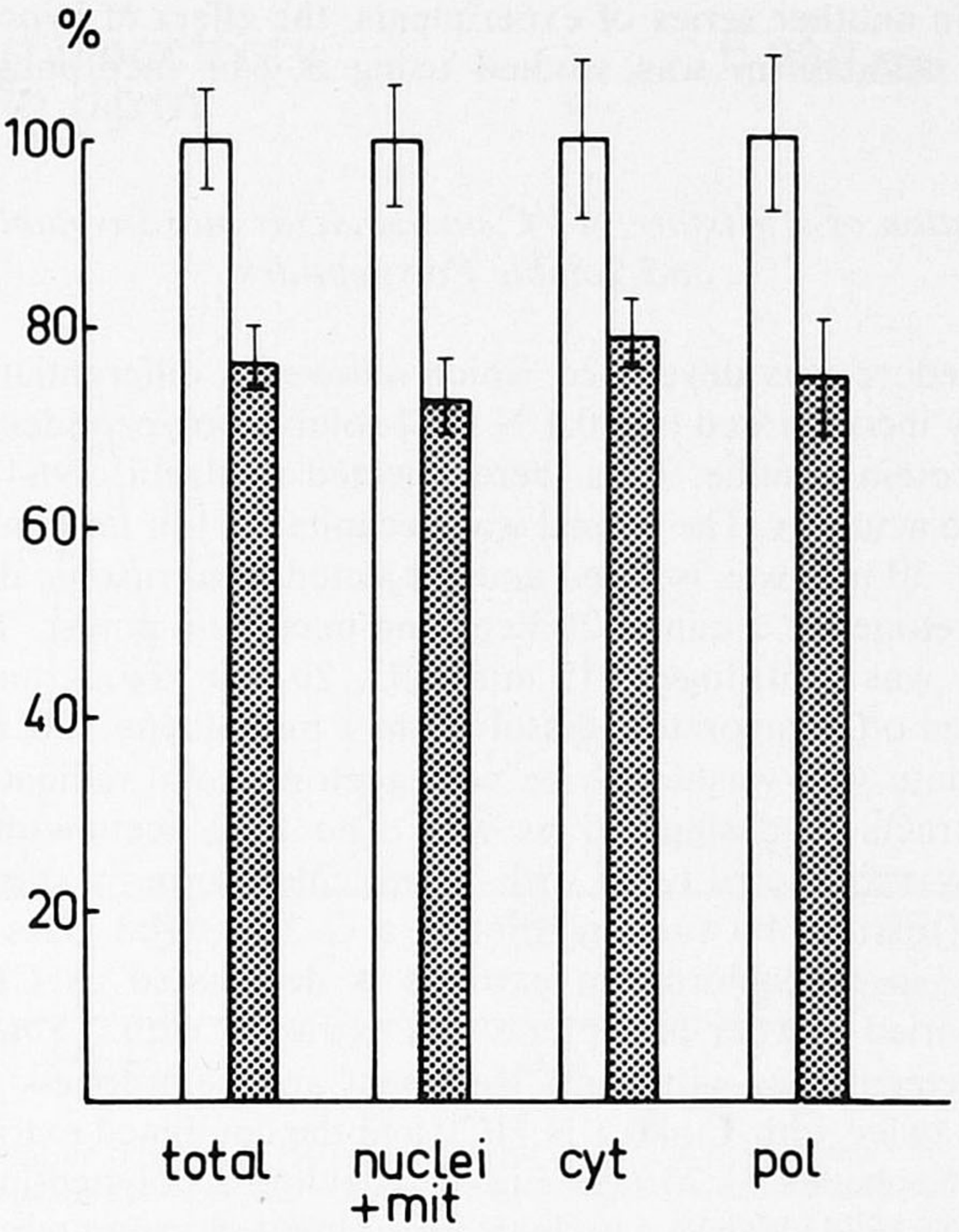

FIG. 3. Effect of hypophysectomy on incorporation of (4,5-^{3}H)-leucine into rat brainstem proteins during an incorporation period of 5 min.

The acid-insoluble radioactivities of the isolated cell fractions were divided by the radioactivity of the acid-soluble precursor pool. Values obtained from hypophysectomized rats in this way were expressed as percent of the values obtained from intact rats.

Open columns: intact rats; mean ± SEM; n = 10. *Stippled columns:* hypophysectomized rats; mean ± SEM; n = 8. mit = mitochondrial fraction; cyt = postmitochondrial cytoplasmic fraction; pol = polysomes. p values (t-test) for hypophysectomized rats: *total*, $0.0005 < p < 0.001$; *nuclei + mit*, $0.001 < p < 0.005$; *cyt* and *pol*, $0.01 < p < 0.025$.

At later times, there appeared to be a net loss of radioactivity. This is in good agreement with the results of Lajtha and Toth (1961) who also reported a rapid loss of administered amino acids from the brain. Moreover, it was found that 5 min after (^{3}H)-leucine injection, brainstem polysomes were labeled with high specific activity (dpm/μg protein), indicating an optimal labeling of growing peptide chains. Usually an incorporation period of 1 hr is used in order to obtain optimal labeling of overall cell protein *in vivo*.

Therefore, in another series of experiments, the effect of hypophysectomy on protein metabolism was studied using a 1-hr incorporation period.

Incorporation of a Mixture of ^{14}C-amino Acids into Insoluble Proteins and Soluble Polypeptides

A procedure was developed which allowed a differentiation between radioactivity incorporated into 0.1 N HCl-soluble polypeptides and into the insoluble protein residue. Rats were injected (Valzelli, 1964) with 5 μC U-^{14}C-amino acid mix. The animal was decapitated 1 hr later, and the brainstem (440 $\pm$ 30 mg) was isolated and subjected to ultrasonic disintegration in 7.5 ml acetone for 1 min at 20 kcps and maximum power. The resulting homogenate was centrifuged (45 min, 4°C, 20,000 $\times$ g_{av}), and the supernatant poured off, evaporated, dissolved in 1 ml Soluene-100, and counted. The precipitate was washed twice with acetone; total radioactivity in the acetone extracts is designated as AE. The final acetone-dried powder precipitate was extracted twice with 7.5 ml chloroform by stirring 1 hr and filtering the mixture by suction through a G-2 sintered glass filter. Total radioactivity in the chloroform extracts is designated as CE. The final chloroform-dried powder precipitate was extracted with 2.5 ml 0.1 N HCl by the aforementioned ultrasonic treatment and centrifuged. The residue was washed twice with 1 ml 0.1 N HCl, and the combined extracts were gel filtrated on Sephadex G 25 (see Fig. 4), yielding a polypeptide peak PEP and an amino acid peak AA, which were counted separately. The 0.1 N HCl-insoluble proteinous residue PR was dissolved in 2 ml Soluene-100 and counted. The yields of radioactivity in the various fractions are expressed as percentages of the total recovery $\pm$ SD. Radioactivity was measured in 10 ml of scintillation solution, consisting of toluene and Triton-X 100 (11:18, v/v) and containing 4 g 2,5-diphenyloxazole (PPO) per liter. Counting efficiencies were determined by the channels ratio method.

The combined radioactivity in AE, CE, and AA was regarded to represent the precursor pool; control experiments in which the amino acid mixture was added to the brainstem homogenate of non-injected animals as well as 0-hr incorporation experiments revealed that 98% of the total recovered radioactivity was present in these three fractions. The combined radioactivity in PEP and PR was used as a measure for incorporated radioactivity. For eight different incorporation periods, ranging from 0 to 17 hr, there was close agreement between data obtained by this method and values obtained by homogenization of the brainstem in 5% trichloroacetic acid (TCA) and counting the TCA-soluble respectively the TCA-insoluble frac-

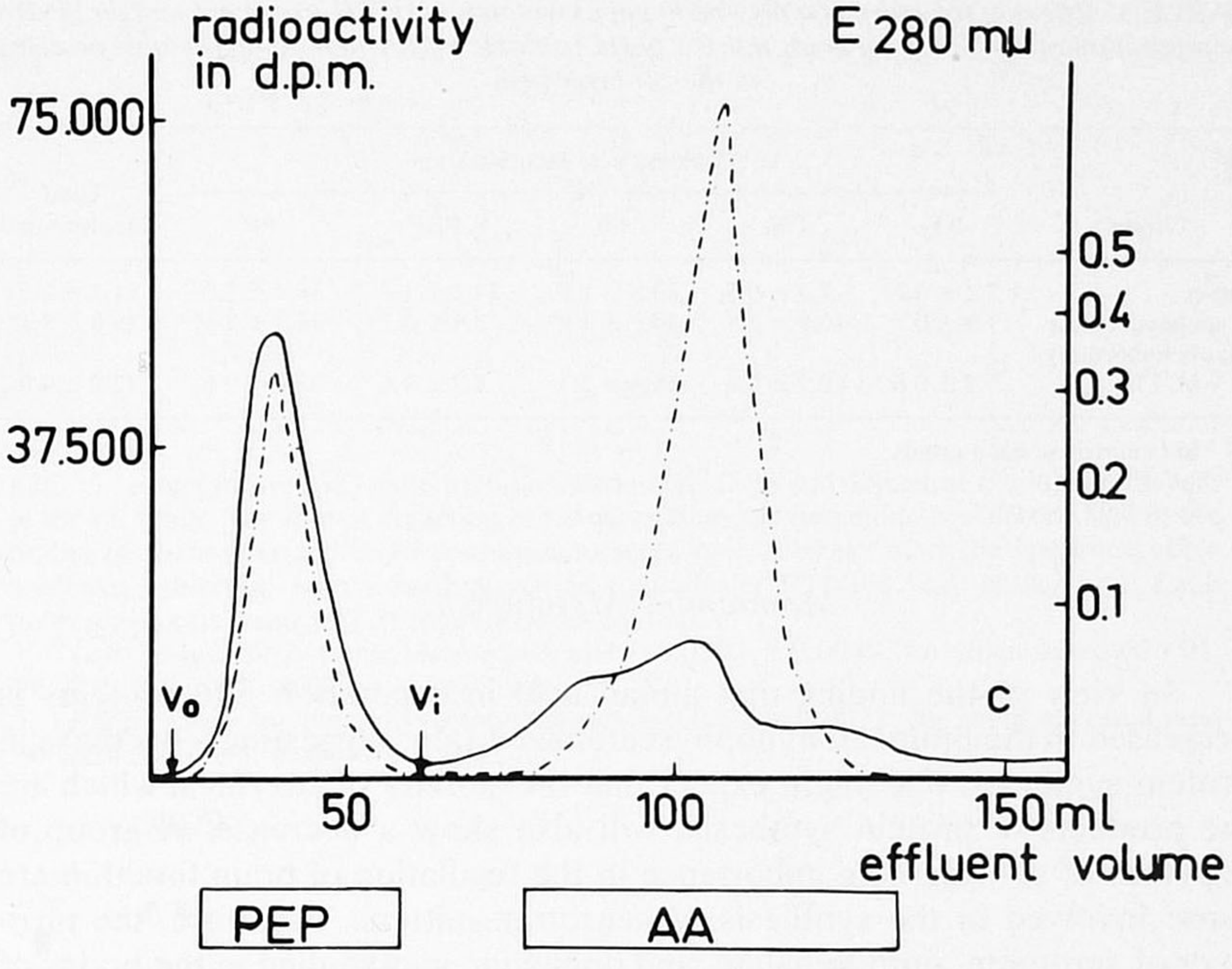

FIG. 4. Separation of a 0.1 N HCl extract of rat brainstem on Sephadex G 25. Separation was carried out on a 1.9 × 70 cm Sephadex G-25 column, equilibrated with 0.1 M acetic acid. Elution was performed with the same solvent at a rate of 12 ml/hr, and fractions of 1 ml were collected. V_0 and V_i indicate the theoretical values for void and internal volume.

–.–.–.–.– = radioactivity; _____ = extinction at 280 mμ.

tions. The former procedure has the advantage of discriminating between 0.1 N HCl-insoluble proteins, of which virtually all radioactivity is solubilized by incubation with pronase, and 0.1 N HCl-soluble polypeptides, which remain open for further investigation. When this procedure was applied to compare 1-hr incorporation of the ^{14}C-amino acids into proteins and polypeptides in the brains of intact rats and of rats hypophysectomized 3 weeks before, a significant difference was found in incorporation of radioactivity into both polypeptides and proteinous residue (Table 3). It is interesting to note that hypophysectomy leads to a decrease in incorporation which is particularly pronounced in the labeling of 0.1 N HCl-soluble polypeptides. The incorporation into this PEP-fraction is obviously more affected by hypophysectomy than by incorporation into the insoluble proteinous residue.

TABLE 3. *Effect of hypophysectomy and hypophysectomy + ACTH treatment on 1-hr in vivo incorporation of U-^{14}C-amino acids into 0.1 N HCl-soluble polypeptides and insoluble proteins in the rat brainstem*

Group	U-^{14}C-amino acid incorporation					Total incorporated
	AE	CE	AA	PEP	PR	
Intact	7.2 ± 0.4	7.2 ± 0.5	33.8 ± 1.9	14.1 ± 1.7	36.7 ± 3.2	51.3 ± 2.6
Hypophysectomy	7.9 ± 0.6	10.9 ± 1.4	40.7 ± 1.9[a]	7.9 ± 0.7[a]	31.4 ± 2.6[b]	39.8 ± 3.1[a]
Hypophysectomy +$ACTH_{4-10}$	7.2 ± 0.6	10.7 ± 0.8	39.1 ± 3.1	8.7 ± 1.6	33.0 ± 2.6	42.2 ± 4.0

Six animals in each group.
[a] $p < 0.0005$.
[b] $p < 0.02$ (t-test).

Monoamine Metabolism

In view of the finding that amino acid incorporation into proteins is decreased in the brains of hypophysectomized rats, suggesting a decrease in protein synthesis, one might expect that the activity of enzymes, which are the products of protein synthesis, will also show a decrease. A group of enzymes of considerable importance in the regulation of brain function are those involved in the synthesis of neurotransmitters. Therefore, the turnover of serotonin, noradrenaline, and dopamine was studied in the brains of hypophysectomized rats.

Serotonin turnover was studied by measuring the increase in brain levels of serotonin and the decrease in brain levels of 5-hydroxyindole acetic acid (5-HIAA) following monoamine oxidase inhibition by tranylcypromine (10 mg/kg, i.p.), according to Neff and Tozer (1968). Noradrenaline and dopamine turnover rates were determined by measuring the decrease in brain levels of these amines after inhibition of the enzyme tyrosine hydroxylase with α-methyltyrosine methylester (H44/68). Spectrofluorimetric techniques were used to determine serotonin and 5-HIAA (Bogdanski, Pletscher, Brodie, and Udenfriend, 1956), and also noradrenaline and dopamine (Taylor and Laverty, 1968).

The turnover rates of serotonin, noradrenaline, and dopamine were determined in the brain of female intact rats, weighing 140 to 150 g, and of hypophysectomized and sham-operated rats, operated when weighing 140 to 150 g. Turnover rates were measured 2 and 3 weeks following the operation. No significant differences were observed in the steady-state levels of the three amines and 5-HIAA in the various groups.

The turnover rates of the three amines in the brains of sham-operated rats, both 2 and 3 weeks after the operation, did not differ from those in the

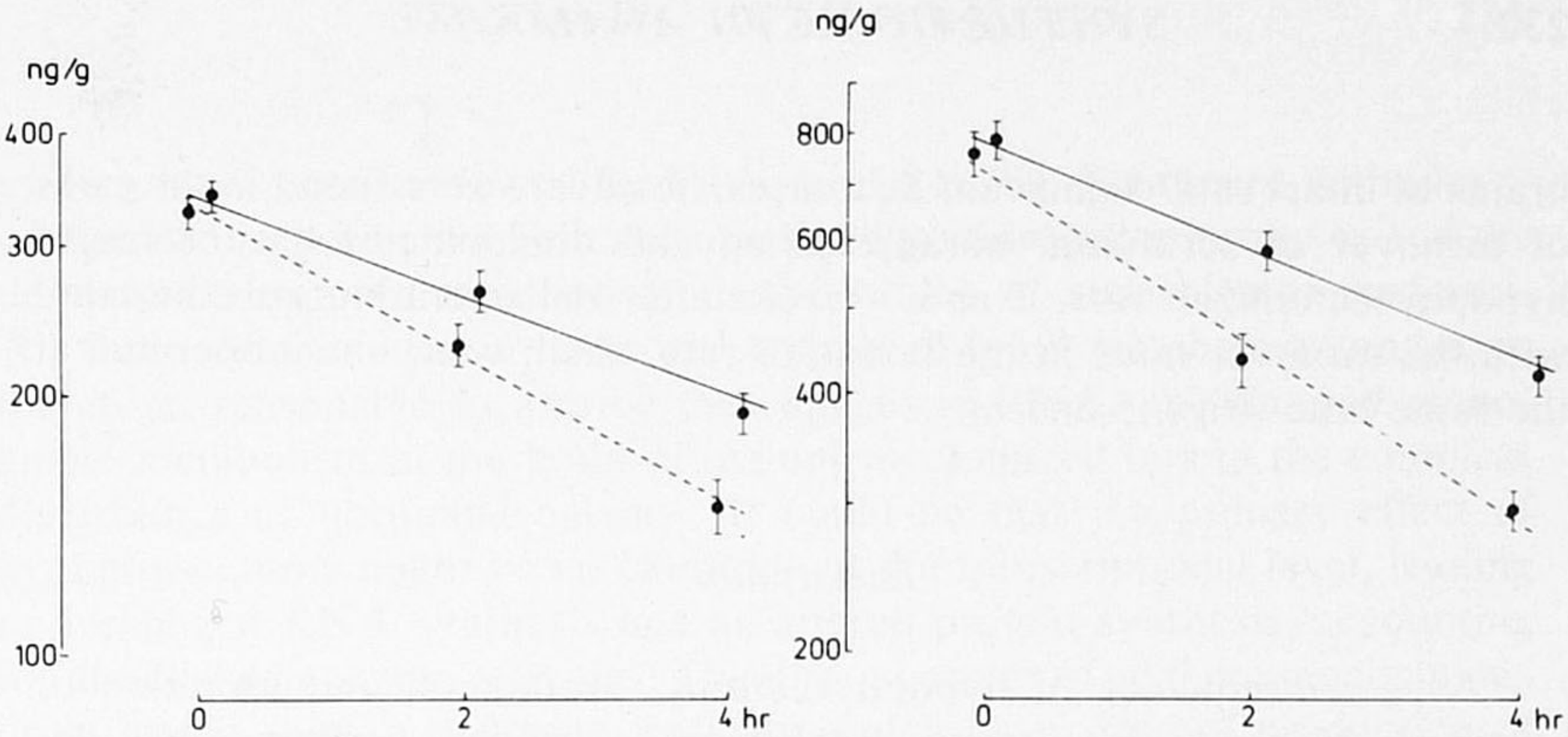

FIG. 5. Effect of hypophysectomy on noradrenaline (*left*) and dopamine (*right*) metabolism in the rat brain. The rate of depletion of noradrenaline and dopamine was determined in the brains of sham-operated and hypophysectomized rats, 3 weeks after the operation, after synthesis inhibition with α-methyltyrosine methylester (H44/68, 250 mg/kg i.p.). Each point represents mean $\pm$ SEM for at least six animals.

Left: Slope for hypophysectomized rats, -0.0703 ± 0.0080; for sham operated rats, -0.0939 ± 0.0081. ($0.025 < p < 0.05$.)

Right: Slope for hypophysectomized rats, -0.0728 ± 0.0073; for sham operated rats, -0.1024 ± 0.0084. ($0.01 < p < 0.02$.)

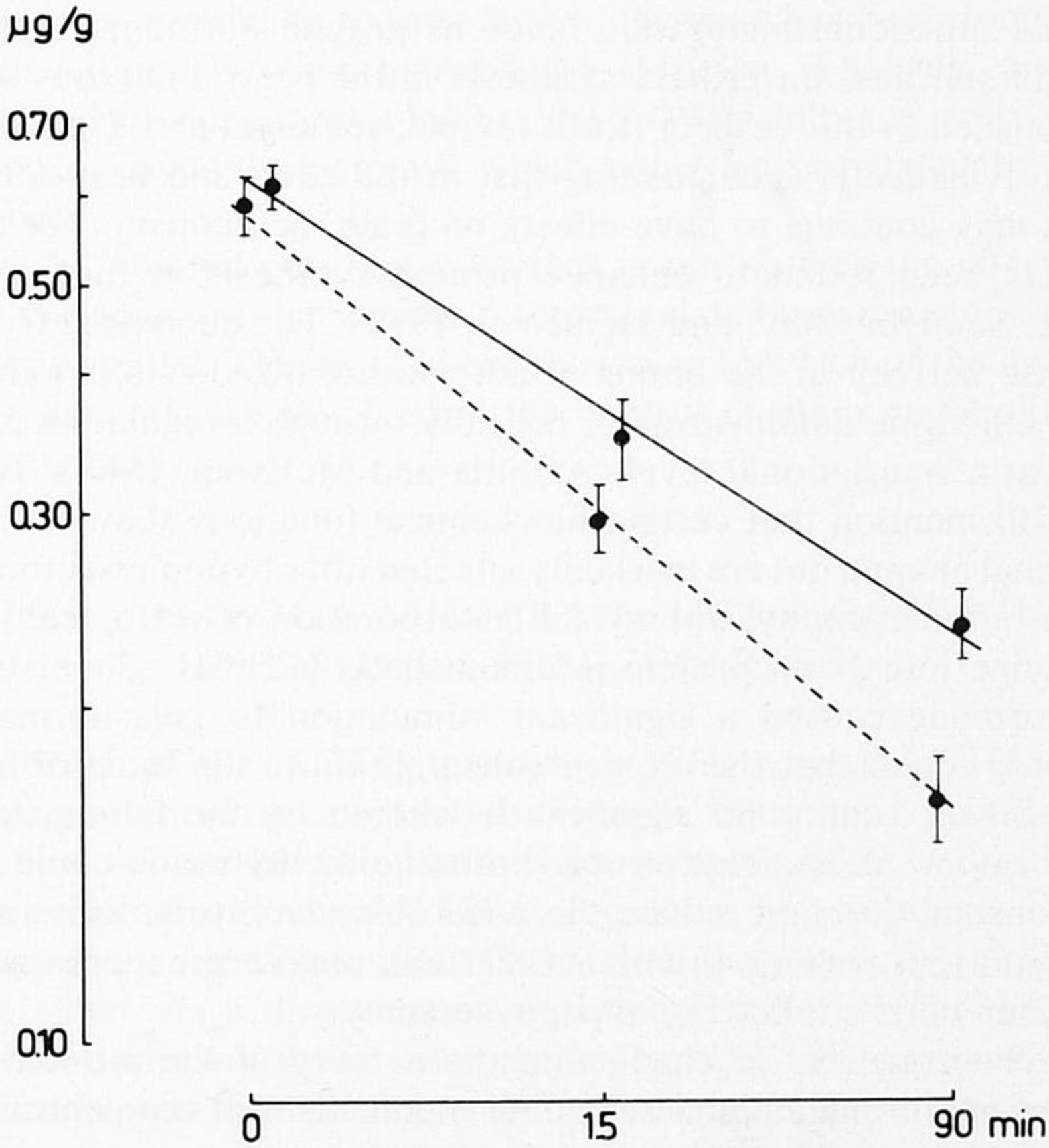

FIG. 6. Effect of hypophysectomy on serotonin metabolism in the rat brain. The rate of depletion of 5-HIAA was determined in the brains of sham-operated and hypophysectomized rats, 3 weeks after the operation, after inhibition of monoamine oxidase with tranylcypromine (10 mg/kg, i.p.). Each point represents the mean $\pm$ SEM for at least six animals. Slope for hypophysectomized rats, -0.0045 ± 0.0004; for sham operated rats, -0.0063 ± 0.0004. ($0.001 < p < 0.005$.)

brains of intact rats. Significant decreases, however, were found in the rates of turnover of serotonin, noradrenaline, and dopamine in the brains of hypophysectomized rats, 2 and 3 weeks after the operation, as compared with the turnover rates in the brains of rats which were sham-operated at the same time (Figs. 5 and 6).

Discussion

As a consequence of hypophysectomy, production and secretion of hormones by the target organs of the pituitary trophic hormones will decrease. These hormones and the hormones of the pituitary itself are known to act as regulators of metabolic processes in specific tissues, and participate as such in the homeostasis of the organism as a whole. Several of the pituitary hormones and the hormones in their target organs have been found to exert effects on metabolic processes in the central nervous system. Hormone actions on protein metabolism in the developing brain have received particular attention. The regulatory role of hormones capable of promoting growth and functional maturation (such as growth hormone, thyroid hormones, and steroids) on protein synthesis in the central nervous system is well established in this respect (for a review, see Geel and Timiras, 1970). However, evidence is accumulating that in the adult and near-adult brain, hormones may continue to have effects on brain metabolism. For instance, ACTH has been found to enhance protein synthesis in the adult brain (Jakoubek, Semiginovský, and Dědičová, 1971). The decreased tryptophan hydroxylase activity in the brains of adrenalectomized rats is partially restored by cortisone administration, possibly through a regulation of protein synthesis at a translational level (Azmitia and McEwen, 1968). Takahashi et al. (1970) mention that certain biochemical functions show no response in the normal animal, but are markedly affected after hypophysectomy. They found that after hypophysectomy the incorporation of isotopically labeled phenylalanine into brain protein is diminished, and that administration of growth hormone caused a significant stimulation to near-normal levels. Reiss (1961) found that the oxygen consumption in the brain of hypophysectomized rats cannot be significantly altered by the administration of growth hormone alone. Thyrotropic hormone or thyroxine could increase oxygen consumption and reduce the anaerobic glycolysis. Reiss and Rees (1961) found that treatment with ACTH can reduce the increased ^{32}P uptake by brain cortex following hypophysectomy.

The consequences of changes in the activity of the pituitary-adrenal system for noradrenaline and serotonin metabolism in the central nervous

system have been reviewed by Fuxe et al. (1970). Treatment with glucocorticoids did normalize both the increased noradrenaline turnover and the decreased serotonin metabolism in the brains of adrenalectomized rats. All these data indicate a hormonal control of brain metabolism, and it is, therefore, reasonable to ascribe the changes in RNA, protein, and monoamine metabolism in the brain of hypophysectomized rats to the complete disturbance of hormonal balance. It could be that the primary effect of hypophysectomy might be an alteration at the transcriptional level, leading to a changed RNA synthesis and an altered protein synthesis, accounting for diminished enzyme activities. The turnover rates of all three monoamines investigated show a decrease, indicative of a lower rate of production of synthesizing enzymes. Adrenalectomy has been shown to lead to an increase in central noradrenaline metabolism and a decrease in that of serotonin (Fuxe et al., 1970). Hypophysectomy, however, brings about a decrease in the turnover rates of both amines. Therefore, it seems likely that the effect of hypophysectomy on monoamine metabolism is due to a direct action of one or more of the pituitary hormones on brain metabolism and not to a lower rate of secretion of glucocorticoids from the adrenal cortex. In this respect it is interesting to note that a decrease has been found in the activities of adrenal tyrosine hydroxylase (Mueller, Thoenen, and Axelrod, 1970) and of dopamine-β-hydroxylase (Weinshilboum and Axelrod, 1970) following hypophysectomy, which could be prevented by ACTH administration.

It must be pointed out, however, that in our studies the effects of replacement therapies on the various altered metabolic parameters have not yet been investigated. Therefore, a conclusion as to which of the hormones, alone or in concert, are involved in the control of brain metabolism is as yet not warranted.

ACTH ANALOGS AND BRAIN METABOLISM IN HYPOPHYSECTOMIZED RATS

The effects of the ACTH analogs $ACTH_{1-10}$ and $ACTH_{4-10}$ were studied because there is substantial evidence that these peptides affect conditioned avoidance behavior in hypophysectomized as well as in intact rats (see Introduction). These ACTH analogs have no appreciable endocrine or systemic effects (de Wied, 1969). It is more likely that they exhibit their behavioral effect via a direct action on brain structures. This hypothesis received extra support from experiments by van Wimersma Greidanus (1971), who showed that not only subcutaneous administration of the pep-

tides, but also direct implantation into the brain resulted in resistance to extinction (van Wimersma Greidanus and de Wied, 1971). Localization studies revealed that in particular the centrum medianum in the diencephalon is sensitive to the behavioral influence of the peptides. The locus of action of the peptides in a circumscript area of the brain suggests the existence of a receptor site in this brain area for an amino acid sequence shared by ACTH and α- and β-MSH. This receptor site appears to be different from the receptor site for ACTH in the adrenal cortex. Hofmann, Wingender, and Finn (1970) found that although a particulate fraction from beef adrenal cortex tissue did bind isotopically labeled $ACTH_{1-20}$, it failed to bind radioactively labeled $ACTH_{1-10}$.

In the biochemical experiments reported here, similar amounts of the ACTH analogs were administered for the same period of time as used in the behavioral studies in order to determine a possible relation between behavioral and neurochemical phenomena. These peptides were administered as long-acting zinc phosphate preparations in a dose of 20 μg s.c. each 48 hr, generally during a 13-day period.

In previous experiments it had been found that treatment with $ACTH_{1-10}$, in combination with shuttle-box conditioning of hypophysectomized rats, increased the content of polysomes (Gispen, de Wied, Schotman, and Jansz, 1971). However, no effect of the peptide treatment *per se* on polysome patterns could be detected. From this and other experiments, it was concluded that this biochemical alteration in the brainstems of hypophysectomized rats, which acquired the response as a result of peptide treatment, was related to an interaction of the peptide treatment with the process of acquisition (Gispen et al., 1971).

The aim of the experiments reported in this chapter was to find a first cue with which to unravel the mechanism of action of $ACTH_{1-10}$ and $ACTH_{4-10}$ at a neurochemical level. Treatment with $ACTH_{1-10}$ or $ACTH_{4-10}$ failed to affect polysome patterns, uridine incorporation into various RNA fractions, and turnover of serotonin, noradrenaline, and dopamine in the brain of hypophysectomized rats. However, a significant increase in labeled leucine incorporation into rapidly labeled proteins was observed in the brainstems of hypophysectomized rats treated with $ACTH_{1-10}$. The *in vivo* incorporation of 4,5-^{3}H-leucine into proteins 5 min after injection was measured as described above, the day after the last injection of the $ACTH_{1-10}$ preparation.

Brainstems of an $ACTH_{1-10}$-treated or a placebo-treated hypophysectomized rat were homogenized, and the homogenate was fractionated as described. The radioactivities in the acid-soluble precursor pool and in the acid-insoluble proteins were calculated as part of the total recovery. A

28% ($p < 0.01$) increase in the incorporation of leucine into the acid-insoluble fraction was found as a result of treatment with $ACTH_{1-10}$ (Fig. 7). Although there was a similar tendency in the 1-hr labeled amino acid incorporation into protein, no significant effect could be demonstrated in this respect. Pilot experiments suggest that ^{3}H-leucine incorporation into proteins 5 min after injection is diminished when $ACTH_{1-10}$-7-D-phenylalanine is administered.

Thus, it is obvious that $ACTH_{1-10}$ exhibits a specific effect on metabolism in the brain of the hypophysectomized rat, which runs parallel

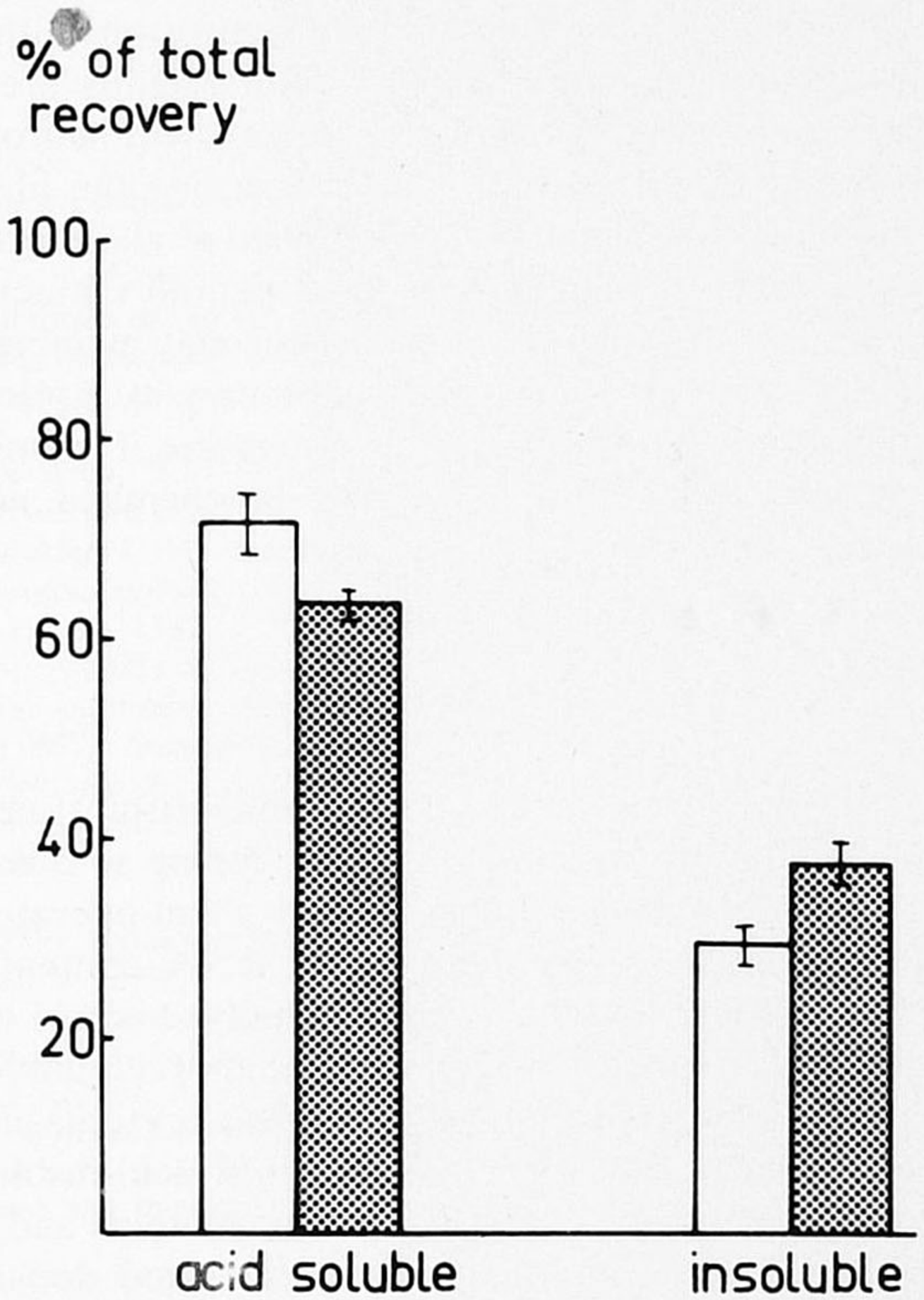

FIG. 7. Effect of treatment with $ACTH_{1-10}$ on (4,5-^{3}H)-leucine incorporation into proteins in the rat brainstem, during a 5-min incorporation period. From the brainstem homogenates, the total acid-insoluble and the total soluble fractions were isolated and assayed for the incorporated radioactivity as described in the text. Values obtained were expressed as percent of total recovered radioactivity in the homogenates

Open columns: placebo treatment (mean ± SEM, n = 6). *Stippled columns:* $ACTH_{1-10}$ treatment (mean ± SEM, n = 6); $0.005 < p < 0.01$.

with the behavioral effect. It appears that $ACTH_{1-10}$ exerts this influence on brain metabolism at a translational level. Other endocrine and metabolic disturbances seem to be unaffected. Jakoubek et al. (1971) reported an increased brain protein synthesis *in vivo* and *in vitro* as a result of ACTH administration. Our data with the ACTH analog $ACTH_{1-10}$ are in good agreement with this observation and might imply that ACTH exerts this effect via an extra-adrenal mechanism.

As stated before, it was concluded from implantation experiments that specific brain areas are sensitive to the behavioral effect of the synthetic ACTH analogs. At present we do not know which of the neurotransmitters are involved in this brain system. Influences on neurotransmitter metabolism may become evident when more defined brain regions are investigated.

It is not yet known how the ACTH analogs affect neuronal processes. There are very few data in the literature concerning the physiological effects of these peptides (for a review, see de Wied et al., *in press*). Summarizing this review, one might suggest that these peptides affect neurophysiological parameters via an interaction with neuronal membranes, possibly implying an involvement in the formation of new synaptic connections.

Further studies are in preparation to determine if membrane interactions are indeed involved in the observed biochemical and behavioral effects of $ACTH_{1-10}$ and $ACTH_{4-10}$.

SUMMARY

The effects of hypophysectomy on the metabolism of neuromolecules and monoamines in the rat brain were studied for up to 3 weeks after the operation. When compared with intact rats or sham-operated rats, hypophysectomized animals showed a decrease in RNA content in the brainstem; in polysome content, especially in large polysomes in the brainstem; in incorporation of ^{3}H- and ^{14}C-uridine into acid-precipitable material; in incorporation of ^{3}H- and ^{14}C-uridine into nuclear, cytoplasmic, and polysomal RNA; in incorporation of ^{3}H-leucine into acid-insoluble proteins; in incorporation of a ^{14}C-amino acid mixture into proteins and polypeptides; and in turnover rates of serotonin, noradrenaline, and dopamine. No differences were found in total protein content or steady-state levels of serotonin, 5-HIAA, noradrenaline, and dopamine.

Hypophysectomy reduces the rat's ability to acquire a shuttle-box avoidance response. This deficient behavior of hypophysectomized rats can be restored by treatment with ACTH analogs. To determine what relationship might exist between macromolecular metabolism in the brain and

behavior, the influence of the ACTH analogs $ACTH_{1-10}$ and $ACTH_{4-10}$ on the respective biochemical parameters was investigated. No effects could be detected after chronic treatment of hypophysectomized rats with these peptides on polysome patterns, on ^{3}H- and ^{14}C-uridine incorporation into RNA, on incorporation of ^{14}C-amino acids into proteins and polypeptides, or on the turnover rates of serotonin, noradrenaline, and dopamine. However, a 28% increase was found in ^{3}H-leucine incorporation into rapidly labeled proteins in the brains of hypophysectomized rats after treatment with $ACTH_{4-10}$.

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