BRAIN STEM POLYSOMES AND AVOIDANCE PERFORMANCE OF HYPOPHYSECTOMIZED RATS SUBJECTED TO PEPTIDE TREATMENT

W. H. GISPEN*, D. DE WIED, P. SCHOTMAN AND H. S. JANSZ

Rudolf Magnus Institute for Pharmacology, and Laboratory for Physiological Chemistry, Medical Faculty, University of Utrecht, Utrecht (The Netherlands)

(Accepted May 5th, 1971)

INTRODUCTION

There is considerable evidence indicating that hormones of the pituitary–adrenal system are important determinants of active avoidance behaviour[20,21]. Moreover, hormones of this system may affect electrophysiological and biochemical processes in the brain[22]. Several years ago it was demonstrated that removal of the pituitary causes a marked impairment of the performance of rats in the acquisition of a shuttle-box avoidance response[18]. Treatment of these rats with synthetic ACTH-like peptides restored their deficient performance almost to normal levels[20]. A number of experiments have suggested that these peptides affect avoidance behaviour in a specific way and presumably via a neurotropic mechanism[6,20,21,24].

The present study was an attempt to relate avoidance conditioning of hypophysectomized rats to chemical events in the brain stem. For this reason profiles of polysomes isolated from the brain stem were selected for study and used as a parameter of macromolecular metabolism. This decision was based on recent work suggesting that RNA and protein metabolism in the brain are involved in acquisition or consolidation of newly learned behaviour[7]. Moreover, as reported previously[5], hypophysectomy itself markedly alters macromolecular metabolism in the brain. One of these changes is a reduction of the polysome content of the brain stem. It was therefore of interest to study brain polysomes in relation to the acquisition performance of hypophysectomized rats.

MATERIALS AND METHODS

Animals and surgery

Male albino Wistar rats of an inbred strain, weighing 160–180 g, were used.

* Address for reprint requests: W. H. Gispen, Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands.

Hypophysectomy was performed via the transauricular route under light ether anaesthesia. Macroscopic examination of the sella turcica, loss of body weight, and adrenal atrophy were indications that the surgery had been performed correctly.

Injected materials

Long acting zinc phosphate suspensions[19] of the following peptides were used: synthetic $ACTH_{1-10}$ (20 μg/0.5 ml) and a peptide fraction isolated from hog anterior pituitary coded BC-15-3d[13,23]. Placebo suspensions were zinc phosphate preparations without the peptide.

Shuttle-box conditioning

The acquisition of a conditioned avoidance response (CAR) was studied in a shuttle-box, as described previously[18]. The conditioned stimulus (CS) was a buzzer presented 5 sec before the unconditioned stimulus (US) of electric shock (0.2 mA). The rat could avoid the shock by crossing a barrier between the two compartments. Ten trials were presented in a session of 10 min on a variable interval schedule. The training lasted 10 days with one session per day. A rat met the acquisition criterion when 8 CARs were scored in 10 consecutive trials.

Treatment

After surgery (on day 1) the animals were subjected to one of the following procedures: (a) the rats stayed in their home cages up to day 18; (b) they were left in their home cages and were injected from day 7 on for 10 days every other day either with placebo suspension or peptide suspension (0.5 ml, subcutaneously); (c) this procedure was an extension of procedure b, the rats being conditioned for 10 days in the shuttle-box beginning on the day after the first injection (day 8).

Section

On day 18 (the day after the last acquisition session) the rats were killed by decapitation. To determine possible effects of the peptides on endocrine tissue, the adrenals, testes and thymus were dissected, cleaned and weighed. For the same purpose corticosterone levels in the blood plasma were determined by the method of Van der Vies *et al.*[17].

Tissue fractionation and preparation of polysomes

Brain stem. Three brain stems from similarly treated rats were pooled, and polysomes were obtained by the method described previously[5]. The material obtained by this method has been characterized as polysomes[5] and marking of the monosomes in the profiles is based on previous results.

Liver. Liver polysomes were obtained by a procedure that differed from the previously described method for brain polysomes[5] in the following steps. Livers were dissected, and each liver was cut into pieces and homogenized in the homogenization medium (1 : 2, w/v). The post-mitochondrial supernatant (20 min at 10,000 × *g*)[5] of the homogenate was decanted and shaken for 30 sec with 1/9 vol. of a freshly prepared solution of 10% (w/v) sodium deoxycholate in 0.03 *M* Tris–HCl (pH 8.2). Polysomes were isolated from a 5 ml aliquot of this mixture as described for brain stem polysomes[5].

Analysis of polysomes

The isolated polysomal pellets were suspended and layered on top of a 27 ml linear 15–30% sucrose gradient (see Gispen *et al.*[5]). The gradients were centrifuged at 63,000 × *g* for 2.5 h in an IEC ultracentrifuge B35 (Rotor SB 110). In a second experiment the gradients were centrifuged at 63,000 × *g* for 2.5 h in a Spinco ultracentrifuge L2–65 (Rotor SW 25.1). After centrifugation the absorbance pattern of the separated polysomes was measured continuously at 260 nm in a Vanguard spectrophotometer. The absorbance pattern was divided into 3 regions[5]: those of monosomes, of small polysomes and of larger polysomal aggregates. The mean absorbances of these regions were obtained from the patterns.

RESULTS

Peptide treatment, brain stem polysomes and acquisition

In the first experiments hypophysectomized rats were subjected to treatment procedure c. They were treated with $ACTH_{1-10}$, BC-15-3d or placebo suspension, and conditioned for 10 days in a shuttle-box. As shown in Fig. 1, the peptide-treated rats

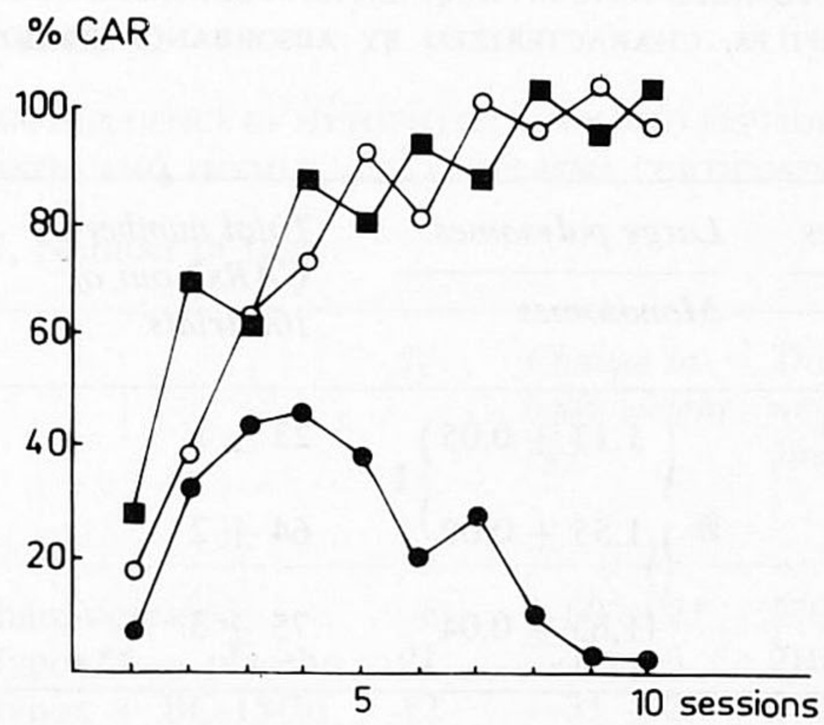

Fig. 1. The influence of peptide treatment on the performance of hypophysectomized rats in shuttle-box conditioning. Acquisition training lasted 10 days with one session of 10 trials per day. Mean performance of rats treated with placebo suspension (●, n = 3), with BC–15–3d (■, n = 3) and with $ACTH_{1-10}$ (○, n = 3). n = number of rats.

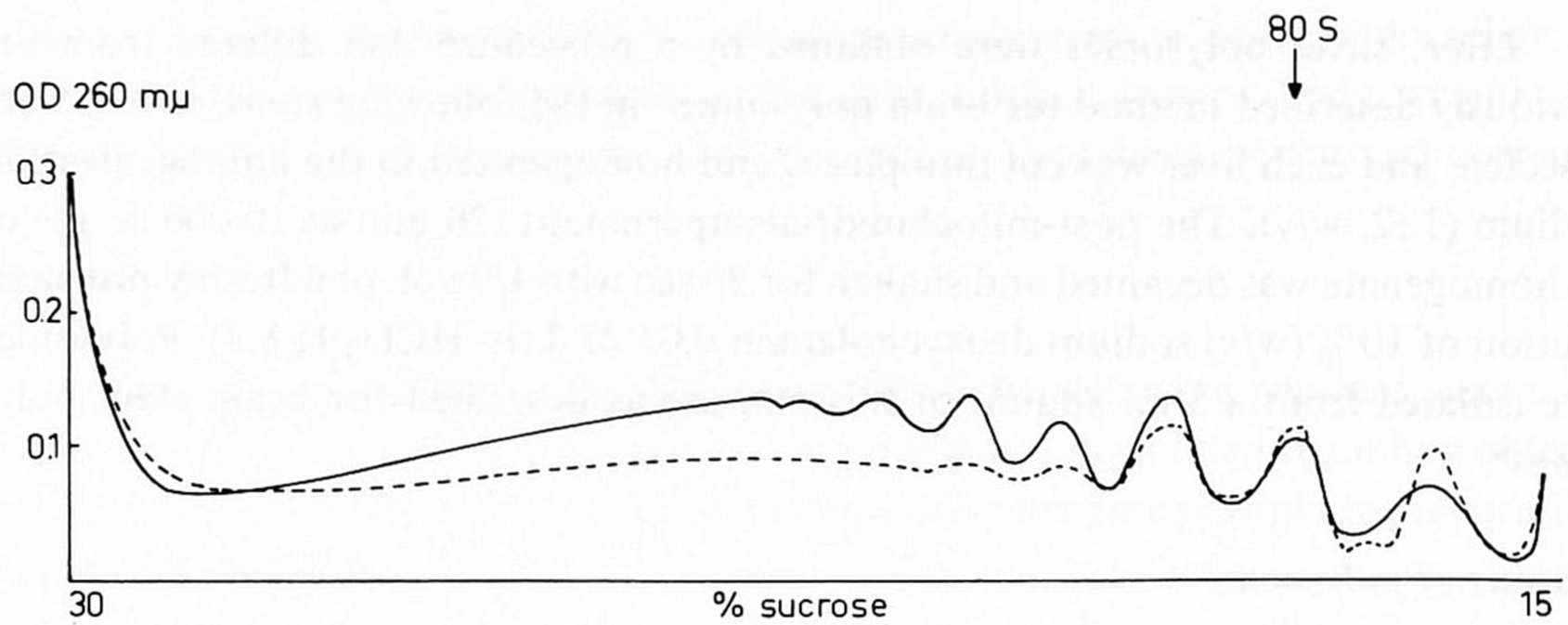

Fig. 2. Polysome profiles obtained from brain stems of hypophysectomized rats treated with placebo or $ACTH_{1-10}$ suspension and conditioned in a shuttle-box (see Fig. 1). Brain stems of 3 similarly treated rats were pooled, and homogenized, and polysomes were isolated. The polysome suspension was layered over a 27 ml 15–30% linear sucrose gradient and centrifuged at 63,000 × *g* for 2.5 h. After centrifugation the absorbance at 260 nm was measured continuously. 80 S refers to the region of the monosomes. Hypophysectomized rats treated with $ACTH_{1-10}$ and conditioned in a shuttle-box (———); hypophysectomized rats treated with placebo suspension and conditioned in a shuttle-box (– – – –).

reached the acquisition criterion within approximately 6 days, in contrast with rats treated with placebo suspension. The effect of $ACTH_{1-10}$ on the avoidance performance is similar to that of $ACTH_{4-10}$. This ACTH analogue is the smallest ACTH-like peptide tested that has a full behavioural effect (*i.e.*, restoration of normal conditioning performance)[20]. The BC-15-3d fraction is a pure peptide fraction, isolated from hog anterior pituitary[13]. Preliminary studies of de Wied *et al.*[23] suggest that the active principle is a peptide of 6–10 amino acids.

TABLE I

THE INFLUENCE OF PEPTIDE TREATMENT OF HYPOPHYSECTOMIZED RATS ON ACQUISITION PERFORMANCE IN A SHUTTLE-BOX AND ON BRAIN STEM POLYSOME PROFILES, CHARACTERIZED BY ABSORBANCE RATIOS

N, Number of groups of 3 rats.

	N	*Small polysomes*	*Large polysomes*	*Total number of CARs* out of 100 trials*
		Monosomes	*Monosomes*	
Hypox** + placebo	7	1.34 ± 0.06***	1.17 ± 0.05 §	23 ± 1
Hypox + BC-15-3d	4	1.41 ± 0.04	§§ 1.55 ± 0.08 §	64 ± 2
Hypox + $ACTH_{1-10}$	3	1.30 ± 0.05	1.63 ± 0.04	75 ± 3

* Conditioned avoidance responses.
** Hypophysectomized.
*** Mean ± S.E.M.
§ $P < 0.05$ (modified *t*-test).
§§ $P < 0.02$ (modified *t*-test).

The day after the last training session, the rats were killed, and polysomes were isolated from their brain stems. In previous studies the material isolated by the method used was clearly characterized as polysomes[5]. As was concluded from the absorbance patterns at 260 nm of the isolated polysome pellets, the patterns obtained from peptide-treated rats differed from those of placebo-treated rats. In Fig. 2 two typical profiles are shown. The profile obtained from brain stems of hypophysectomized rats, all of which had reached the conditioning criterion as a result of treatment with $ACTH_{1-10}$, shows a higher content of larger polysomes than that of placebo-treated hypophysectomized animals. There is no significant difference between the two patterns in respect to the regions of monosomes and small polysomes.

In order to characterize the polysome profiles obtained, the ratios of mean absorbances of the various regions of the profiles were determined[5]. In Table I the ratios of the mean absorbances of the profiles obtained from the various treatment groups are listed. In all instances, there was a significant difference between the ratio of the mean absorbance of the large polysomes to that of the monosomes for the placebo-treated rats and the ratio for the peptide-treated rats. The ratio of the small polysomes to monosomes remained unchanged. Table I also contains measures of acquisition performance. As shown in Fig. 1, the peptide-treated rats made significantly more CARs than placebo-treated rats.

Table II shows data concerning body weight, weight of endocrine organs and plasma corticosterone levels. Data of a group of sham-operated rats are also included. This group, subjected to treatment procedure a, remained in their home cages for 18 days after sham operation. Since Table II shows that organs, directly or indirectly stimulated by the pituitary in the intact rat, were atrophied in the hypophysectomized rats used, the observed difference in polysome profile cannot be the result of a possible ineffectiveness of the surgery. Also, there was no difference in any endocrine parameters between placebo- and peptide-treated rats. Thus Table II shows that these peptides affected avoidance acquisition via an extra-adrenal mechanism of action[6,20,21].

TABLE II

THE INFLUENCE OF HYPOPHYSECTOMY AND PEPTIDE TREATMENT ON BODY WEIGHT, WEIGHT OF ADRENALS, TESTES AND THYMUS, AND ON PLASMA CORTICOSTERONE CONCENTRATION

N, Number of rats.

	N	*Change in body weight (g)*	*Thymus weight (mg)*	*Testes weight (mg)*	*Adrenals weight (mg)*	*Corticosterone (μg/100 ml plasma)*
Sham-operated	6	+60 ± 2*	370 ± 20	2380 ± 56	30.5 ± 1.1	28.2 ± 3.0
Hypox** + placebo	21	—30 ± 3	210 ± 11	630 ± 32	9.3 ± 0.5	4.3 ± 0.8
Hypox + BC-15-3d	12	—35 ± 2	230 ± 17	600 ± 40	10.3 ± 0.4	3.9 ± 1.0
Hypox + $ACTH_{1-10}$	9	—23 ± 3	239 ± 17	502 ± 30	8.0 ± 0.2	4.6 ± 0.9

* Mean ± S.E.M.
** Hypophysectomized.

TABLE III

THE INFLUENCE OF TREATMENT OF HYPOPHYSECTOMIZED RATS WITH $ACTH_{1-10}$ ON BRAIN STEM POLYSOME PROFILES, CHARACTERIZED BY ABSORBANCE RATIOS

N, Number of groups of 3 rats.

	N	*Small polysomes*	*Large polysomes*
		Monosomes	*Monosomes*
Hypox* + Placebo	5	1.32 ± 0.05**	1.60 ± 0.11
Hypox + $ACTH_{1-10}$	5	1.32 ± 0.07	1.65 ± 0.11

* Hypophysectomized.
** Mean ± S.E.M.

Peptide treatment and brain stem polysomes

The possibility exists, however, that the observed change in brain stem polysomes is the result of an interaction of the peptide treatment *per se* with these polysomes. To test this hypothesis, an experiment was carried out in which hypophysectomized rats were subjected to procedure b (see Materials and Methods). In fact, the experimental design is the same as in the first experiment, but instead of being trained, the animals were left in their home cages. The rats were injected with $ACTH_{1-10}$ or with placebo suspension.

In Table III the polysome profiles as characterized by their absorbance ratios are shown. The values for the absorbance ratios in this experiment differ from those of the first experiment, since the polysomes were analyzed under different conditions. The difference in absolute values, however, has no bearing on the relationship between the treatment groups. It is a common finding that changes in procedure can yield different absolute values for a given parameter while maintaining the relative difference between treatment conditions. In other studies from our laboratory and elsewhere (*e.g.*, refs. 1, 4) still other polysome patterns and absolute values were obtained for intact animals. Still, there remained a difference between intact and hypophysectomized animals[5]. In the present instance there was no difference in polysome profile between the peptide- and placebo-treated groups. It was concluded, therefore, that the peptide treatment *per se* had no effect on brain stem polysomes.

Home cage control versus placebo-treated, conditioned animals

The change in polysome pattern observed in the first experiment might have been caused by a different amount of stimulation (CS and US) received by peptide-treated and placebo-treated rats. Peptide-treated rats avoided the US much more than placebo-treated rats. Moreover, it is well established that environmental stimulation itself may affect macromolecular metabolism in the brain[7] and, therefore, also polysomes[3]. On the other hand the change in the profile could reflect a decrease in polysome content in brain stems of placebo-treated rats instead of an increase in the

TABLE IV

THE INFLUENCE OF SHUTTLE-BOX CONDITIONING OF HYPOPHYSECTOMIZED RATS, SUBJECTED TO PLACEBO TREATMENT, ON BRAIN STEM POLYSOME PROFILES, CHARACTERIZED BY ABSORBANCE RATIOS

N, Number of groups of 3 rats.

	N	*Small polysomes* / *Monosomes*	*Large polysomes* / *Monosomes*	*Total number of CARs* out of 100 trials*
Hypox** (home cage)	3	1.16 ± 0.04***	1.08 ± 0.09	—
Hypox + placebo + acquisition training	3	1.24 ± 0.07	1.13 ± 0.11	24 ± 2

* Conditioned avoidance responses.
** Hypophysectomized.
*** Mean ± S.E.M.

content in brain stems of peptide-treated rats. To exclude these possibilities an additional experiment was performed. One group of hypophysectomized rats was subjected to procedure a (see Materials and Methods) in which the rats did not receive injections and were left in their home cages. The other group of hypophysectomized rats received placebo injections and was conditioned in the shuttle-box (procedure c, see Materials and Methods). After the animals had been killed, polysomes were isolated from the brain stem and analyzed. Table IV shows the absorbance ratios of the polysome profiles obtained. There was no difference between the 2 groups in respect to their brain stem polysomes. Thus, the change observed in the first experiment was not the result of differences in stimulation during acquisition training. Furthermore, the change reflects an increase in polysome content in the brain stems of those rats that met the acquisition criterion instead of a decrease in polysome content in the brain stems of those rats that were unable to master the task.

Peptide treatment, liver polysomes and acquisition

These experiments support the hypothesis that the change in brain polysomes is indeed related to brain function. Therefore, it was thought worthwile to obtain some information concerning the specificity of the observed change in polysomes in the brain. Liver was used as a tissue for examination other than brain tissue. Livers were isolated from a number of placebo-treated and from BC-15-3d-treated rats which were all used in the first experiment. Polysomes were isolated from liver and subsequently analyzed on linear sucrose gradients. The isolated polysomes showed no contamination with the protein ferritin, since the collected gradient fractions did not show any absorption at 320 nm[9]. As can be seen in Fig. 3, the liver polysome pellets contained relatively more small polysomes and monosomes than larger polysomal aggregates. This phenomenon in livers of hypophysectomized rats is also reported by several other authors[11,15,16]. As already mentioned, hypophysectomy *per se* has a

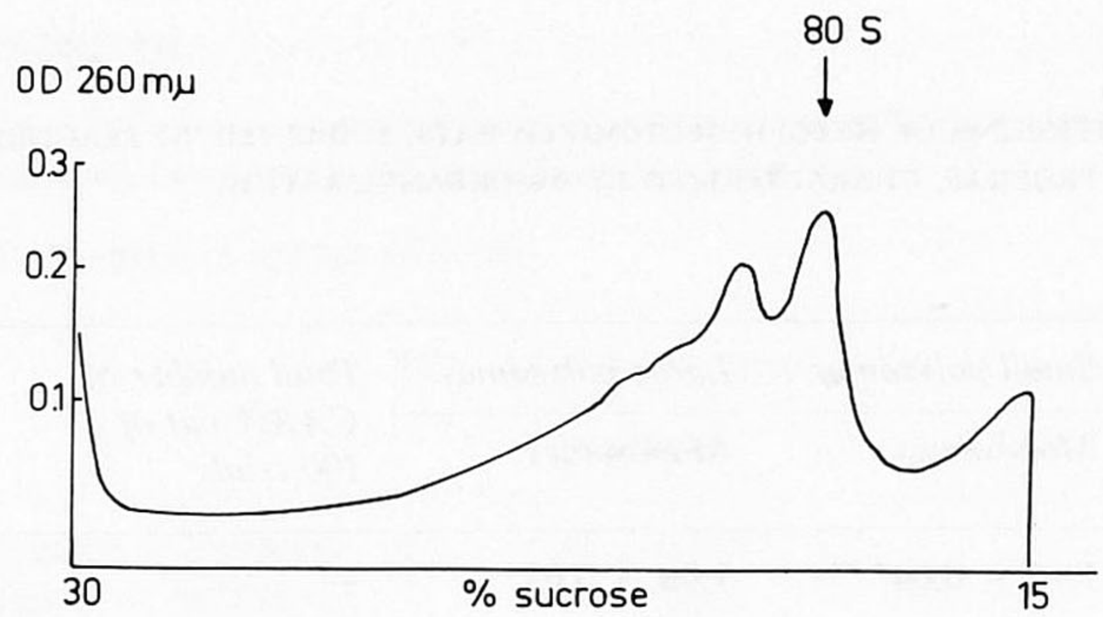

Fig. 3. Polysome profile obtained from the liver of a hypophysectomized rat treated with placebo suspension and conditioned in a shuttle-box (see Fig. 1). The liver was cut into pieces and homogenized. Polysomes were isolated and their suspension was layered over a 27 ml 15–30% linear sucrose gradient, followed by centrifugation at 63,000 × *g* for 2.5 h. After centrifugation the absorbance at 260 nm was measured continuously. 80 S refers to the region of the monosomes.

TABLE V

THE INFLUENCE OF TREATMENT OF HYPOPHYSECTOMIZED RATS WITH THE PEPTIDE FRACTION BC-15-3d IN COMBINATION WITH SHUTTLE-BOX CONDITIONING, ON LIVER POLYSOME PROFILES, CHARACTERIZED BY ABSORBANCE RATIOS

N, Number of rats.

	N	*Small polysomes*	*Large polysomes*
		Monosomes	*Monosomes*
Hypox * + placebo	3	0.85 ± 0.09**	0.36 ± 0.04
Hypox + BC-15-3d	4	0.88 ± 0.05	0.37 ± 0.04

* Hypophysectomized.
** Mean ± S.E.M.

similar influence on polysomes in the brain stem[5]. However, no effect of shuttle-box conditioning in combination with peptide treatment was found on the polysome content in the liver (see Table V). Accordingly, the observed change in polysome profiles obtained from brain stems of peptide-treated hypophysectomized rats which acquired the CAR seems to be specific for the brain.

DISCUSSION

The present work describes a relationship between acquisition of a conditioned avoidance response in hypophysectomized rats and changes in brain stem polysomes. Hypophysectomized rats were treated with placebo or peptide suspensions ($ACTH_{1-10}$, BC-15-3d) and conditioned in a shuttle-box (Fig. 1). Afterwards, polysomes were isolated from their brain stems. The peptide-treated rats appeared to acquire the response while placebo-treated rats did not. This acquisition ran parallel with an increase of 34.7 and 39.3% respectively in absorbance in the region of large polysomes

(Fig. 2, Table I). At least in the case of $ACTH_{1-10}$, the change could not be the result of peptide treatment itself (Table III) and this difference in polysome profile was not found in liver (Table V). Furthermore, the difference in polysome profile between the two treatment groups was not caused by an alteration in the profile of the placebo-treated rats but was indeed an increase in absorbance in the profile of the peptide-treated rats which acquired the conditioning criterion (Table IV). It is unlikely that the difference in absorbance in the region of large polysomes was caused by substances (*e.g.*, membranes) other than RNA. The same result could be obtained by collecting the gradients into fractions, measuring their UV spectra and calculating the RNA content of the fractions from the E_{260}/E_{232} ratio[12]. The observed increase in polysome content was not the result of difference in environmental stimulation but seemed related to the acquisition itself (Table IV).

The increase in polysome content might reflect an improved binding of *m*-RNA to ribosomes in the cytoplasm. Zomzely *et al.*[27] suggest a relation between nerve–cell activity and aggregation to polysomes. However, more evidence is necessary before such a conclusion is justified. On the other hand the increase in polysomes could reflect a situation in which more *m*-RNA and ribosomes are present in the cytoplasm as a result of an increased synthesis. For example, Glassman and his colleagues clearly demonstrated that conditioning of mice in a jump-out box resulted in an increase in labelled uridine incorporation into polysomal RNA, which was primarily located in brain stem areas[1,2,8,10,25,26]. Also, Dellweg *et al.*[4] found an increase of 34% in rat brain polysomes as a result of acquisition of new behaviour. Thus the present results corroborate those of others. The functional significance of such changes remains to be determined.

In hypophysectomized rats the disturbance of RNA metabolism in the brain may reflect, as it does for peripheral tissue such as liver, the lack of pituitary hormones and/or the lack of hormonal activity of target organs of the pituitary. The deficient performance of hypophysectomized rats may well be the result of changes in the organism other than that in brain stem RNA metabolism. Neverthesless, the increase in polysome content reported in this study might be interpreted as the result of stimulation of brain stem neurones caused by the acquisition process itself via a permissive action of the peptide. Such a stimulation of brain metabolism during conditioning has been reported by several others[7]. The observed change in polysomes is probably not specific to the information being stored, but may, as Shashoua[14] suggested for RNA metabolism in goldfish brain, be required for consolidation processes in general. Learning and environmental stimulation may have in common that both under certain experimental conditions[7] affect brain metabolism in terms of hyperactivity of the metabolism in the circuits involved. Further experiments are needed to elucidate the action of the peptides on brain macromolecular metabolism.

SUMMARY

The deficient avoidance conditioning performance of hypophysectomized rats was restored to a normal level following treatment with the ACTH-like peptide

$ACTH_{1-10}$, or with a peptide fraction isolated from pig pituitary. In contrast to placebo-treated animals, the increase in conditioned avoidance responses among the peptide-treated rats was associated with an increase in the polysome content of their brain stems. For $ACTH_{1-10}$, at least, the change in brain polysomes was not caused by the peptide treatment itself, nor could it be demonstrated in liver. Moreover, the increase could not be attributed to differences in the environmental stimulation experienced by the various treatment groups. It appeared that the increase in brain stem polysomes in the hypophysectomized animals related to an interaction between peptide treatment and the process of acquisition of a conditioned avoidance response.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. S. Lande, Dr. A. Witter and to Organon Co., Oss, The Netherlands for supplying the peptides.

The authors also wish to thank Dr. R. Ader for stimulating discussions during the preparation of the manuscript.

REFERENCES

1 ADAIR, L. B., WILSON, J. E., AND GLASSMAN, E., Brain function and macromolecules. III. Uridine incorporation into polysomes of mouse brain during short term avoidance conditioning, *Proc. nat. Acad. Sci. (Wash.)*, 60 (1968) 606–613.
2 ADAIR, L. B., WILSON, J. E., AND GLASSMAN, E., Brain function and macromolecules. IV. Uridine incorporation into polysomes of mouse brain during different behavioural experiences, *Proc. nat. Acad. Sci. (Wash.)*, 60 (1968) 917–922.
3 APPEL, S. H., DAVIS, W., AND SCOTT, S., Brain polysomes: response to environmental stimulation, *Science*, 157 (1967) 836–838.
4 DELLWEG, H., GERNER, R., AND WACKER, A., Quantitative and qualitative changes in ribonucleic acids of rat brain dependent on age and training experiences, *J. Neurochem.*, 15 (1968) 1109–1119.
5 GISPEN, W. H., WIED, D. DE, SCHOTMAN, P., AND JANSZ, H. S., Effects of hypophysectomy on RNA metabolism in rat brain stem, *J. Neurochem.*, 17 (1970) 751–761.
6 GISPEN, W. H., WIMERSMA GREIDANUS, TJ. B. VAN, AND WIED, D. DE, Effects of hypophysectomy and $ACTH_{1-10}$ on responsiveness to electric shock in rats, *Physiol. Behav.*, 5 (1970) 143–146.
7 GLASSMAN, E., The biochemistry of learning; an evaluation of the role of RNA and protein, *Ann. Rev. Biochem.*, 38 (1969) 605–646.
8 GLASSMAN, E., AND WILSON, J. E., The effects of short term experiences on the incorporation of uridine into RNA and polysomes of mouse brain. In D. DE WIED AND J. A. W. M. WEIJNEN (Eds.), *Pituitary, Adrenal and the Brain, Progress in Brain Res., Vol. 32*, Elsevier, Amsterdam, 1970, pp. 245–249.
9 JACKSON, R. J., MUNRO, A. J., AND KORNER, A., Errors in the use of 260 mμ absorbation for estimation of RNPr particles, *Biochim. biophys. Acta (Amst.)*, 91 (1964) 666–668.
10 KAHAN, B. E., KRIGMAN, M. R., WILSON, J. E., AND GLASSMAN, E., Brain function and macromolecules. VI. Autoradiographic analysis of the effect of a brief training experience on the incorporation of uridine into mouse brain, *Proc. nat. Acad. Sci. (Wash.)*, 65 (1970) 300–308.
11 KORNER, A., Regulation of the rate of synthesis of messenger ribonucleic acid by growth hormone, *Biochem. J.*, 92 (1964) 449–456.
12 MUNRO, H. N., AND FLECK, A., The determination of nucleic acids, *Meth. biochem. Anal.*, 14 (1966) 113–176.
13 LANDE, S., LERNER, A. B., AND UPTON, G. V., Isolation of new peptides related to β-melanocyte stimulating hormone, *J. biol. Chem.*, 240 (1965) 4259–4263.
14 SHASHOUA, V. E., RNA metabolism in goldfish brain during acquisition of new behavioral patterns, *Proc. nat. Acad. Sci. (Wash.)*, 65 (1970) 160–167.

15 STAEHELIN, M., Effect of hypophysectomy on rat liver polyribosomes, *Biochem. Z.*, 342 (1965) 459–468.
16 TATA, J. R., AND WILLIAMS-ASHMAN, H. G., Effects of growth hormone and triiodo thyroxine on amino acid incorporation by microsomal subfractions from rat, *Europ. J. Biochem.*, 2 (1967) 366–374.
17 VIES, J. VAN DER, BAKKER, R. F. M., AND WIED, D. DE, Correlated studies on plasma corticosterone and on adrenal steroid formation rate *in vitro*, *Acta Endocr. (Kbh.)*, 34 (1960) 513–523.
18 WIED, D. DE, The influence of the anterior pituitary on avoidance learning and escape behavior, *Amer. J. Physiol.*, 207 (1964) 255–259.
19 WIED, D. DE, Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats, *Proc. Soc. exp. Biol. (N.Y.)*, 122 (1966) 28–32.
20 WIED, D. DE, Effects of peptide hormones on behavior. In W. F. GANONG AND L. MARTINI (Eds.), *Frontiers in Neuroendocrinology*, Oxford Univ. Press, New York, 1969, pp. 97–140.
21 WIED, D. DE, DELFT, A. M. L. VAN, GISPEN, W. H., WEIJNEN, J. A. W. M., AND WIMERSMA GREIDANUS, TJ. B. VAN, The role of the pituitary-adrenal system hormones in active-avoidance conditioning. In S. LEVINE (Ed.), *Hormones and Behavior*, Academic Press, New York, in press.
22 WIED, D. DE, AND WEIJNEN, J. A. W. M. (Eds.), *Pituitary, Adrenal and the Brain, Progress in Brain Research, Vol. 32*, Elsevier, Amsterdam, 1970, Section I, II and III.
23 WIED, D. DE, WITTER, A., AND LANDE, S., Anterior pituitary peptides and avoidance acquisition of hypophysectomized rats. In D. DE WIED AND J. A. W. M. WEIJNEN (Eds.), *Pituitary, Adrenal and the Brain, Progress in Brain Research, Vol. 32*, Elsevier, Amsterdam, 1970, pp. 213–220.
24 WIMERSMA GREIDANUS, TJ. B. VAN, AND WIED, D. DE, Effects of systemic and intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior, *Neuroendocrinology*, 7 (1971) 291–301.
25 ZEMP, J. W., WILSON, J. B., AND GLASSMAN, E., Brain function and macromolecules. II. Site of increased labeling of RNA in brains of mice during a short term training experience, *Proc. nat. Acad. Sci. (Wash.)*, 58 (1967) 1120–1125.
26 ZEMP, J. W., WILSON, J. B., SCHLESINGER, K., BOGGAN, W. O., AND GLASSMAN, E., Brain function and macromolecules. I. Incorporation of uridine into RNA of mouse brain during short term training experience, *Proc. nat. Acad. Sci. (Wash.)*, 55 (1966) 1423–1431.
27 ZOMZELY, C. E., ROBERTS, S., GRUBER, C. P., AND BROWN, D. M., Cerebral protein synthesis. II. Instability of cerebral messenger ribonucleic acid–ribosome complexes, *J. biol. Chem.*, 243 (1968) 5396–5409.