

Pituitary-Adrenal System, Learning and Performance: Some Neurochemical Aspects

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INTRODUCTION

The role hormones play in the regulation of cell metabolism is well documented. Numerous results suggest that hormones are in a similar way important to brain cell metabolism. Since cell metabolism underlies cell functioning, it seems logical to expect that chemical events in the brain also underly behavioral processes. Furthermore, assuming that hormones do interact with brain cell metabolism, it is then of interest to study the interrelationship of the three factors mentioned: hormones, brain metabolism and behavior. The present paper is an attempt to describe the results of our own work on this interrelationship, within the framework of current views.

HYPOPHYSECTOMY AND AVOIDANCE LEARNING

Removal of the pituitary or adenohypophysis interferes with avoidance learning in shuttle-box conditioning (Applezweig and Baudry, 1955; Applezweig and Moeller, 1959; De Wied, 1964, 1969). It has been suggested that this result is due to the poor physical condition of hypophysectomized rats and/or to an overall deterioration of behavior. Hypophysectomy does of course produce dramatic changes throughout the body in systems which are normally under the influence of the pituitary (Tepperman, 1962). However, there is evidence that argues against the suggestion that a poor physical condition is the basic cause of the impaired avoidance performance. First, recent experiments showed that hypophysectomized (hypox) rats' behavior does not deteriorate in a novel environment. By observing the behavior of hypox and sham-operated rats in a wire-mesh cage with glass front for 5 min, it was found that hypox rats were more actively engaged in exploratory behavior and climbed and jumped more on the wire-mesh sides than did sham-operated rats (Gispen *et al.*, in press). The rats appeared to be capable of making all bodily movements normally involved in performing the avoidance response in shuttle-box conditioning. That such is true also follows from the observation that hypox rats were all able to escape but

not to avoid the foot-shock as the unconditioned stimulus (UCS) in shuttle-box conditioning.

Another study showed that the ability to detect or to respond to the electric foot-shock was certainly not impaired in hypox rats. Hypox rats responded to lower shock intensities of inescapable foot-shock (duration of 1 sec) than did controls (Gispén *et al.*, 1970a). Moreover, treatment of hypox rats with peptides derived from corticotrophin (ACTH), (*e.g.* ACTH₄₋₁₀, ACTH₁₋₁₀) throughout the training period restored their performance to nearly normal levels. Further, under similar conditions, the isomer ACTH₄₋₁₀ (7-D-Phe) had no, or even an inhibiting, effect on the avoidance learning of hypox rats (De Wied, 1969). Since ACTH₄₋₁₀ does not show any endocrine or systemic effect, this important finding suggests that a specific rather than a non-specific mechanism underlies the poor avoidance learning of hypox rats (De Wied, 1969; De Wied *et al.*, 1972). That is, while ACTH₄₋₁₀ had no detectable endocrine or systemic effects, it was sufficient to restore avoidance performance. Thus, although a total hormonal replacement therapy also improved the avoidance performance of hypox rats (De Wied, 1964), it appears that the mechanism of ACTH₄₋₁₀ avoidance improvement is more specific and is not accomplished *via* widespread effects on bodily functions. Further, neither adrenalectomy nor substitution of adrenal steroids in hypox rats affected avoidance conditioning (Appelzweig and Moeller, 1959; Bohus and Endröczy, 1965; De Wied *et al.*, 1968, 1972).

Considerable evidence supports the hypothesis that this specific mechanism of ACTH analogs is related to an action of the peptides on central nervous structures. For example, electrophysiological and neuroendocrine research has shown that ACTH, and in some instances its *N*-terminal sequences, are able directly to influence various aspects of nerve cell functioning, including neuronal firing (Sawyer *et al.*, 1968; Krivoy, 1970; Steiner, 1970; Van Delft and Kitay, 1972), electroencephalogram synchronization (Endröczy *et al.*, 1970), ACTH release (Motta *et al.*, 1965) and macromolecular metabolism (see below). The work of Van Wimersma Greidanus and De Wied (1971) strongly supports the concept that ACTH-like peptides regulate avoidance behavior *via* a neurotrophic mechanism of action. They found that in intact rats both subcutaneous and intracerebral administration of the peptide retards extinction of avoidance learning. Recently, a naturally occurring peptide (an analog of vasopressin) was isolated from hog pituitaries and found to possess potent effects on shuttle-box learning in hypox rats (De Wied *et al.*, 1970; Lande *et al.*, 1971). It has been postulated, therefore, that removal of the pituitary would deplete the animal of neurotrophic peptides which in normal circumstances might play an integrating role in the adaptation of the animal to its environment.

HYPOPHYSECTOMY AND MACROMOLECULAR METABOLISM

Periphery

As mentioned before, removal of the pituitary leads to dramatic disorders in a variety

of tissues and organs. Such findings are consistent with the concept that protein metabolism in the mammalian cell is under hormonal control (growth hormone, thyroxine). After hypophysectomy the overall impression of the events in the peripheral cell is that the lack of hormones leads to a marked limitation of the cytoplasmic machinery involved in protein synthesis (Cardell, 1967, 1970). In hypox animals both the suggested decrease in RNA synthesis (Korner, 1964, 1965; Gupta and Talwar, 1968) as well as an increase in RNA breakdown as a result of high RNase activity (Brewer *et al.*, 1969) could account for the reduced polysome content of the cell (Korner, 1964; Staehelin, 1965; Wurfbain-Moolenburgh, 1968). Moreover, there is evidence that hypophysectomy would lead to a defect at the ribosomal level disrupting regular binding capacity to mRNA or to aminocyl-tRNA (Staehelin, 1965; Garren *et al.*, 1967; Korner, 1968, 1969; Liew and Korner, 1969; Barden and Korner, 1969). However, at least in the binding capacity of ribosomes to mRNA, contradictory evidence has been found (Foster and Sells, 1969). Nevertheless, the altered RNA metabolism is likely to modify protein synthesis and, indeed, a marked reduction of incorporation of amino acids into peptide chains was found in hypox rats, concomitant with a reduction in total protein content (Korner, 1968, 1969; Tata and Williams-Ashman, 1967; Herrlich and Lang, 1967; Cheek and Graystone, 1969; Clemens and Korner, 1970).

It appeared that growth hormone treatment alone was sufficient to counteract this deteriorated metabolism of macromolecules in peripheral organs and tissues of the hypox rat (Tata, 1968; O'Malley, 1968; Brewer *et al.*, 1969; Clemens and Korner, 1970; McDonald and Korner, 1971). In contrast with the detailed studies on the effect of hypophysectomy on macromolecules in the periphery, scattered data are present on its effect on brain macromolecules.

Brain

From neuroendocrine studies it is clear how complex the interaction between hormones and the brain can be (Yates *et al.*, 1971). Because of this complexity, neurochemical correlates of hypophysectomy have often appeared to be inconsistent. Critical points to be considered are the age at which the surgery has been performed, the brain region studied, the time between surgery and biochemical analysis, and, very importantly, an appropriate test of the effectiveness of the surgery. For instance, it has been reported that oxygen consumption of brain cortex does not change as a result of hypophysectomy, while anaerobic glycolysis increases by more than 100% (Reiss, 1961). On the other hand, other investigators find a significant increase of oxygen uptake in certain parts of the hypothalamus after hypophysectomy (Libertun *et al.*, 1969; Moguilevsky *et al.*, 1970). Furthermore, De Vellis and Inglish (1968) have reported that glycerophosphate dehydrogenase (EC. 1.1.1.8) was reduced especially in brain stem tissue, and predicted a lower level of anaerobic glycolysis. The reported decrease in RNA, DNA and protein content of the brain of juvenile rats (Cheek and Graystone, 1969) suggests that there is a similar atrophy in brain and liver in young hypox rats. Indeed, some anatomical changes have been reported to take place

(Diamond, 1968), but it has also been suggested that the young hypox rat dies, because after hypophysectomy the skull (which has ceased to grow) cannot provide the still growing brain with enough space (Geel and Timiras, 1970). The above-cited evidence illustrates the confusion mentioned before. Regarding the metabolism of macromolecules, indications are present that in the brain both RNA (De Vellis and English, 1968; Cheek and Graystone, 1969) and protein metabolism (Dunn and Korner, 1966; Takahashi *et al.*, 1970) are influenced by hypophysectomy. Previous and current work of our group deals with macromolecular aspects of the effect of hypophysectomy on the brain.

In all our work the experimentals are male rats analyzed three weeks after hypophysectomy. In agreement with preliminary observations, it was found that total cell RNA in the brain was reduced as a result of hypophysectomy. Gross topographical localization of the effect indicated that the decrease was confined to brain stem areas. A more detailed analysis gave more insight into the regional effect of hypophysectomy. Total cell RNA was measured and expressed as mg RNA per mg DNA. The highest RNA/DNA ratio was found in the cortex cerebri (*ca.* 2.15) and hippocampus (2.21), whereas the cerebellum (0.42) and bulbus olfactorius (0.95) showed the lowest values. Hypophysectomy reduced the RNA/DNA ratio by *ca.* 20% in the thalamus, hypothalamus, mesencephalon and medulla oblongata. There was also a small but significant reduction in the rostral part of the cortex cerebri (Gispen *et al.*, 1972).

On the basis of these findings it was decided to use the brain stem to study the change in macromolecular metabolism in greater detail. First, an attempt was made to trace the decrease in total RNA down to the subcellular level. After fractionation of brain stem homogenates into nuclei, crude mitochondria, microsomes and post-microsomal supernatant (Gispen *et al.*, 1970b), the only fraction that showed a significant decrease in RNA content as a result of hypophysectomy was that of the microsomes (−20%) (Versteeg *et al.*, 1972). Moreover, isolating polysomes from the post mitochondrial supernatant showed that hypophysectomy caused an absolute decrease in the amount of polyribosomal aggregates containing 3 or more ribosomes (Gispen *et al.*, 1970; Gispen and Schotman, 1970; Schotman *et al.*, in prep.). Additionally, it was found that hypophysectomy reduced the incorporation of uridine into rapidly labeled messenger-like RNA and into ribosomal RNA (Gispen *et al.*, 1970b; Schotman *et al.*, in prep.; Schotman, 1971) isolated from brain stem tissue. Whether or not such a decrease reflected a decrease in RNA synthesis remains to be shown, since an increased breakdown could also account for most of the observed phenomena. Despite this uncertainty, it seems clear that in the brain stem of hypophysectomized rats there is a modification of RNA metabolism leading to a reduced capacity of the RNA machinery to synthesize proteins. However, overall protein content in the brain stem seems unaltered by removal of the pituitary (De Vellis and English, 1968; Schotman, 1971). The only change observed was a decrease of protein in the polysomal pellet obtained from the brain stem homogenate which is in good agreement with the above data obtained from RNA distribution.

By studying the incorporation of [³H]leucine into proteins over a short period of time (5 min), it was found that hypophysectomy led to a decrease of incorporated

radioactivity of *ca.* 20–27%. The decrease was found both in the nuclear and in the cytoplasmic cell fractions. However, we have good reason to believe that the proteins labeled within 5 min after intracranial application of the precursor, are from cytoplasm only. [Treatment of the nuclear fraction with detergent, leaving the nuclei intact, removed all of the label from this fraction (Schotman, 1971).] Analysis of the polysomal fraction showed that preparations obtained from hypox rats contained less label than similar preparations from control rats (Schotman *et al.*, in prep.; Schotman, 1971). It seems reasonable to assume that this reduction reflects a reduced assemblage of amino acids into polypeptide chains. The overall picture therefore is that hypophysectomy changes RNA metabolism in brain stem areas, which indeed has consequences for protein turnover. Using similar techniques, Witter also found, after a pulse length of 60 min, a reduction of incorporation of a mixture of ^{14}C -labeled amino acids in brain stem peptides and proteins (Versteeg *et al.*, 1972). The large effect of hypophysectomy on protein synthesis on the one hand and the absence of an effect on protein content on the other, suggests that the inhibition of protein synthesis pertains to a relatively small fraction of proteins with a high turnover.

AVOIDANCE LEARNING AND MACROMOLECULAR METABOLISM IN THE BRAIN

Various lines of evidence have suggested that chemical events in the brain are involved in the storage of information. A number of reviews, models and hypotheses can be found in the literature which maintain this view, although the available data are limited and often controversial. Among the various possible experimental approaches to the problem, the method in which the fate of a chemical process in the brain is analyzed during or after acquisition of a new behavior pattern, is one of the most important.

Among the first studies which stressed the role of RNA during acquisition of a new behavior, were those by Hydén and coworkers. They found that rats, after learning to climb a wire to obtain food, showed both an increase in cell RNA and a change in base composition of nuclear RNA in the Deiter's nucleus (Hydén and Egyhazi, 1962, 1963). Transfer of handedness resulted in the increased production of DNA-like RNA in certain motoneurons of the "learning" cortical hemisphere (Hydén and Egyhazi, 1964). Although the original interpretations no longer seem fully valid, Hydén's group deserves credit for having been the first to point towards changes in brain RNA related to acquisition of new behavioral patterns.

The work of Glassman and Wilson (Zemp *et al.*, 1966, 1967; Adair *et al.*, 1968a, b; Coleman *et al.*, 1971) seems to be of major importance. They showed that avoidance training of mice and rats resulted in an increased incorporation of radioactive uridine into brain nuclear RNA and polysomes. In these studies, autoradiographic localization pointed towards the diencephalon as a major area of increased incorporation (Zemp *et al.*, 1967; Kahan *et al.*, 1970). A number of experimental variables were studied to determine the conditions responsible for the observed phenomena. Neither yoked training, random foot-shock treatment nor prior trained performing increased the

uridine incorporation into brain RNA above the level of that in quiet mice. From this evidence, the investigators concluded that "the process of learning a conditioned avoidance seems to be relevant to the chemical change observed in these studies" (Glassman, 1969). Some laboratories have initiated programs in this type of research in recent years. In a way, the work of Dellweg *et al.* (1968) can be viewed as a partial confirmation of Hydén's data. They reported that rats undergoing wire-balancing training contained more brain polysomes than did controls.

Recently, Uphouse reported results similar to those found by the group of Glassman and Wilson. Using a single-label method, she found that uridine was incorporated to a greater extent into brain RNA in trained mice than in yoked or quiet mice. Moreover, an increase in hippocampal polysome content was also detected as a result of jump-box training (Uphouse, 1971; Uphouse *et al.*, 1972). Others have described an increased incorporation of [^3H]cytidine into RNA concomitant with a higher content of nuclear RNA, in rat hippocampal nuclei, as a result of reversal training in a Y-maze spatial discrimination task (Bowman and Strobel, 1969). Also, the incorporation of [^3H]UMP into nuclei of hippocampal and cortical neurons was increased as a result of discrimination training, using foot-shock as the reinforcer (Pohle and Matthies, 1971). It is reported that both 50 trials in one 25-min session, and six daily sessions of 30 trials in shuttle-box conditioning, led to an increase in total cell RNA in the hippocampus of female rats. Random treatment with conditioned and unconditioned stimuli did not change the hippocampal RNA content (Nasello and Izquierdo, 1969).

Another, rather preliminary, paper reported a failure to find a change in labeling of hippocampal RNA resulting from the acquisition of a new behavior (Carreres, 1970). Using not histochemical, but rather advanced biochemical, techniques in the search for a correlation between RNA and behavior, it was reported that during shock-avoidance conditioning the synthesis of a unique RNA species took place based upon DNA-RNA hybridization experiments (Machlus and Gaito, 1969). Recently, some doubts have been raised as to the relevance of the reported change (Von Hungen, 1971). Also, in goldfish, acquisition of new behavior patterns seemed to run parallel with qualitative and quantitative changes in RNA metabolism in the brain (Sashoua, 1968, 1970, 1971). Using light as stimulus, Rose *et al.* (1970) found that imprinting behavior in the young chick triggered an increased RNA synthesis in midbrain and roof (at different times after injection of [^3H]uracil), not attributable to non-specific factors (Horn *et al.*, 1971). The above-cited literature stresses the belief that changes in macromolecule metabolism can run parallel with the storage of newly acquired information. However, it certainly does not give any explanation of the biological meaning or of the exact mechanism of action by which learning affects brain metabolism or *vice versa*.

Nevertheless, on the basis of the evidence that brain RNA is affected by hypophysectomy and by avoidance learning as well, an effort was made to study brain RNA during shuttle-box training of hypox rats subjected to peptide treatment. Hypox rats were treated with ACTH₁₋₁₀ or β -melanocyte-stimulating hormone (MSH) or with desglycinamide-lysine vasopressin (De Wied *et al.*, 1970; Lande *et al.*, 1971), a peptide

isolated from hog pituitaries. On day 7 after surgery, the injection treatment started and lasted throughout the training period which consisted of 10 daily sessions beginning on day 8 after the surgery. After the last training session, the rats were killed and the polysomes of their brain stems were isolated and subsequently analyzed on sucrose gradients (Gispen and Schotman, 1970; Gispen *et al.*, 1970c, 1971). Peptide-treated rats, which mastered the task, contained more brain stem polysomes than those rats treated with placebo suspension and which were therefore unable to acquire the conditioned avoidance response. The ratio of polysomes over monosomes was approximately 1.5–1.6 in the rats that acquired the response, whereas the placebo group showed a ratio of 1.2. In the peptide-treated group, the mean total amount of conditioned avoidance responses out of 100 possible was around 70, and this value was 23 for placebo-treated rats (Gispen *et al.*, 1971). Further study showed that peptide treatment *per se* (at least for ACTH_{1–10}) did not affect brain stem polysome profiles in hypox rats. It is of interest to note that treatment of hypox rats with peptide fractions, isolated from hog pituitary, which did not improve avoidance learning in these rats (De Wied *et al.*, 1970), did not result in the increase of polysomes at the end of the training period (Gispen, unpublished). From a number of studies it is clear that environmental stimulation (see Glassman, 1969) and stress (Jakoubek *et al.*, 1970) are able to trigger changes in brain metabolism which directly or indirectly could account for changes in RNA and protein metabolism. Since hypox rats which remained in their home cages throughout the experiment showed brain polysome profiles similar to placebo-treated rats subjected to avoidance training, it seemed clear that the stimulation of the conditioning procedure as such did not trigger the event (*cf.* yoked training of Zemp *et al.*, 1966).

Others have also paid attention to the pituitary-adrenal system and neurochemical events involved in information storage. Flexner and coworkers found that memory of maze learning in mice is blocked for long periods of time by the intracerebral injection of puromycin one or more days after the training period (Flexner *et al.*, 1967). Bilateral adrenalectomy before training protects memory against this action of puromycin (Flexner and Flexner, 1970). This finding led the authors to believe that high circulation levels of ACTH in their adrenalectomized mice would account for the observed "protection". Although it was reported that ACTH administration to intact mice indeed gave protection against memory blockade caused by puromycin (Flexner and Flexner, 1971), it now seems that this result was most likely due to an impurity in the ACTH preparation used, since highly purified ACTH did not give the effect. However, desglycinamide-lysine vasopressin (the natural pituitary peptide, also used by us) did interfere rather strongly with the suppression of memory by puromycin (Lande *et al.*, 1972).

Glassman and coworkers found no differences between sham-operated and adrenalectomized mice in jump-box conditioning, nor did they see differences between sham-operated and hypox rats in short-term avoidance learning in a runway with elevated platform (Adair *et al.*, 1968b; Coleman *et al.*, 1971). Both experiments were conducted 7 days after the surgery, and under both conditions the increased incorporation of uridine into RNA was found. Assuming that the surgery had been performed correctly

(no evidence in this respect was provided), these data are of great interest in combination with our work. Hypophysectomy impaired long-term avoidance learning, and peptide treatment restored the ability of the animals to acquire the task and this in turn gave rise to the increase of polysomes. In the short-term situation and 7 days after the surgery, hypophysectomy did not interfere with avoidance learning, and accordingly these untreated rats showed the increase in uridine incorporation into RNA.

The primary conclusion appears to be that hypophysectomy does not interfere with all kinds of avoidance learning, but rather specifically with long-term active avoidance. However, preliminary studies showed that hypophysectomy did interfere with short-term avoidance in rats in jump-box conditioning, tested three weeks after the surgery. The effect was more variable and less marked than in long-term shuttle-box conditioning (Gispén, 1970). From a neurochemical point of view it seems reasonable to assume that the impaired metabolism of macromolecules, in parts of the brain involved in avoidance learning, is not able to provide the cell with, or to maintain, a certain end-product important for the consolidation of information acquired over a longer period of time. In subsequent research we attempted more fully to elucidate the role of the peptides in this respect.

The changes in polysomes suggest that protein synthesis is also affected by acquisition processes. Indeed, in recent years it was found that transfer of handedness was related to an increase in synthesis of a brain-specific acid-soluble protein S-100 in certain hippocampal pyramidal cells (Hydén and Lange, 1969, 1970a, b). Moreover, an increased synthesis of glycoproteins is reported to occur both in pigeons (Bochoch, 1968) and in rats (Dunn *et al.*, in press) as a result of training experience. Also, the incorporation of [^3H]leucine into proteins as studied by autoradiographic techniques, is altered in relation to an acquisition of avoidance behavior (Beach *et al.*, 1969). Machlus found that the acquisition of a one-way avoidance resulted in an increased phosphorylation of nuclear acidic proteins in rat brain (Machlus, 1971; Machlus *et al.*, 1973a and b). Current experiments of the same group also indicate that phosphorylation of synaptosomal proteins in mice is increased as a result of acquisition in jump-box training (Dunn *et al.*, in press; Glassman *et al.*, 1972, in press). In so far as polysome profiles can be used as an indication of cell protein synthesis (Henshaw *et al.*, 1971), it is clear that our data fit the concept that acquisition of new behavior is accompanied by changes in brain RNA and proteins.

ACTH ANALOGS AND BRAIN MACROMOLECULAR METABOLISM

That ACTH-like peptides have a role in avoidance learning and performance has been shown before and is the subject of a paper by Greven and De Wied (this volume). In a recent review, Krivoy (1970) stressed as a fundamental hypothesis that certain polypeptides play a role in the nervous system as modulators of nervous activity. ACTH and related peptides are capable of altering transmission in the spinal cord under a variety of conditions (Krivoy, 1970), and, as was pointed out above, a

variety of other processes in central nervous system structures seem to be directly influenced by these peptides. From a biochemical point of view, little, if anything, is known about the effect on central nervous system structures. On the other hand, the mechanism by which ACTH stimulates steroidogenesis in the adrenal cortex and exerts its lipolytic effect on the fat cell is relatively well documented.

The interaction of ACTH with the target cell receptor probably consists of a binding of the hormone to the cell membrane (Hofmann *et al.*, 1970) resulting in an activation of adenylcyclase (Garren *et al.*, 1971; Bär and Hechter, 1969; Sutherland *et al.*, 1965; Rodbell, 1967). This in turn leads to an increase in intracellular cyclic AMP levels and subsequently to an activation of a protein kinase (Jost and Rickenberg, 1971; Garren *et al.*, 1971). With respect to the adrenal cell, it has been proposed that the protein kinase would catalyze the transfer of phosphate from ATP to ribosomes. Therefore, it was postulated that ACTH regulates adrenal protein synthesis at the translational level (Garren *et al.*, 1971).

Until now little effort has been made to determine whether or not ACTH acts on the brain cell in a way similar to that on the peripheral target cell. Because ACTH seems to act on rather specific sites in the brain (Steiner, 1970; Motta *et al.*, 1965; Van Delft and Kitay, 1972), it might be difficult to find effects of ACTH on cyclic AMP formation similar to that in the periphery. Work is in progress to elucidate the ACTH-brain receptor mechanism, although with respect to adenylcyclase the observations of Burkhard and Gey (1968) and of Forn and Krishna (1971) do not look promising. Other investigators have found that ACTH treatment of intact mice does affect protein and RNA synthesis in the central nervous system (Jakoubek *et al.*, 1970, 1971a, b), and an extremely high dose of ACTH, injected 60 or 90 min before killing of the animals, resulted in an inhibition of uridine incorporation into RNA of spinal motoneurons and glial cell nuclei (Jakoubek *et al.*, 1971b). Under similar conditions, enhancement of leucine incorporation into proteins was reported (Jakoubek *et al.*, 1971a). However, protein synthesis in whole brain slices seems to be decreased as a result of ACTH treatment (Jakoubek *et al.*, 1970). Since adequate controls were not presented, it is uncertain whether the changes described should be ascribed to ACTH itself, to the increased circulation of corticosteroids resulting from ACTH treatment, or to a combination of both.

In view of the behavioral effects of ACTH analogs, it seemed worthwhile to investigate whether or not these analogs do indeed alter brain stem macromolecular metabolism in hypox rats, despite the observed absence of an effect on the polysome population. Therefore, we treated hypox rats with peptide or placebo (zinc-phosphate) suspension, a procedure similar to that used in the training experiments reported above. A week after the surgery, the rats were injected for 10 days every other day with either peptide suspension (10 μ g peptide/ 0.5 ml s.c.) or placebo suspension. The day after the last injection, radioactive labeled uridine or leucine was injected into the rats' brain stems, and the incorporation rate of the precursors into RNA and protein respectively were studied. It appeared that ACTH₁₋₁₀ (7-L-Phe) treatment did not affect the incorporation of labeled uridine into nuclear or cytoplasmic RNA after 70 min of incorporation. However, the same peptide stimulated the incorporation of

[^3H]leucine into the acid-insoluble fraction (+ 28%) of both nuclei and cytoplasm after 5 min of incorporation. This fraction represents the same protein fraction whose turnover was affected by hypophysectomy (see above). Under similar conditions it was found that ACTH_{1-10} (7-D-Phe) causes an inhibition of leucine incorporation (−28%) and that ACTH_{11-24} has no effect (Schotman, 1971; Schotman *et al.*, 1972). Work is in progress to characterize the proteins involved and to see whether the observed increased incorporation indeed affects synthesis or is related to uptake and pool phenomena (Reith *et al.*, in press).

These results are therefore of interest because the biochemical effects of these peptides run parallel with their behavioral effects. ACTH_{1-10} (7-D-Phe) has, in both circumstances, an opposite effect to that of the L-form (see also De Wied, 1969). ACTH_{11-24} has an effect neither on avoidance learning nor on brain stem leucine incorporation in hypox rats.

The data with the L-isomer of ACTH_{1-10} are in good agreement with those of Reading and Dewar, who reported a stimulation of glycine and leucine incorporation into brain proteins by treatment of intact rats with ACTH_{4-10} (7-L-Phe). However, under their conditions—incorporation period of 48 h and intact rats—no effect of ACTH_{4-10} (7-D-Phe) was found in this respect (Reading and Dewar, 1971). It is tempting to speculate that the observed neurochemical alterations are indeed related to the action of these peptides on avoidance learning of hypox rats in shuttle-box conditioning.

CONCLUSION AND SUMMARY

Hypophysectomy impaired both avoidance learning and brain-stem macromolecular metabolism in the rat. To study the relationship between the two phenomena, two types of experiment were carried out. First, hypox rats were treated with ACTH-like peptides and trained in a shuttle-box for 10 days. As a result of the peptide treatment, the hypox rats acquired the avoidance task, and showed an increased amount of polysomes in their brain stems. Second, hypox rats were treated with behaviorally active ACTH analogs, one that enhanced avoidance learning of hypox rats (ACTH_{1-10} -7-L-Phe), one that had no effect in this respect (ACTH_{11-24}) and one whose action was rather opposite to that of ACTH_{1-10} (7-L-Phe), namely ACTH_{4-10} (7-D-Phe).

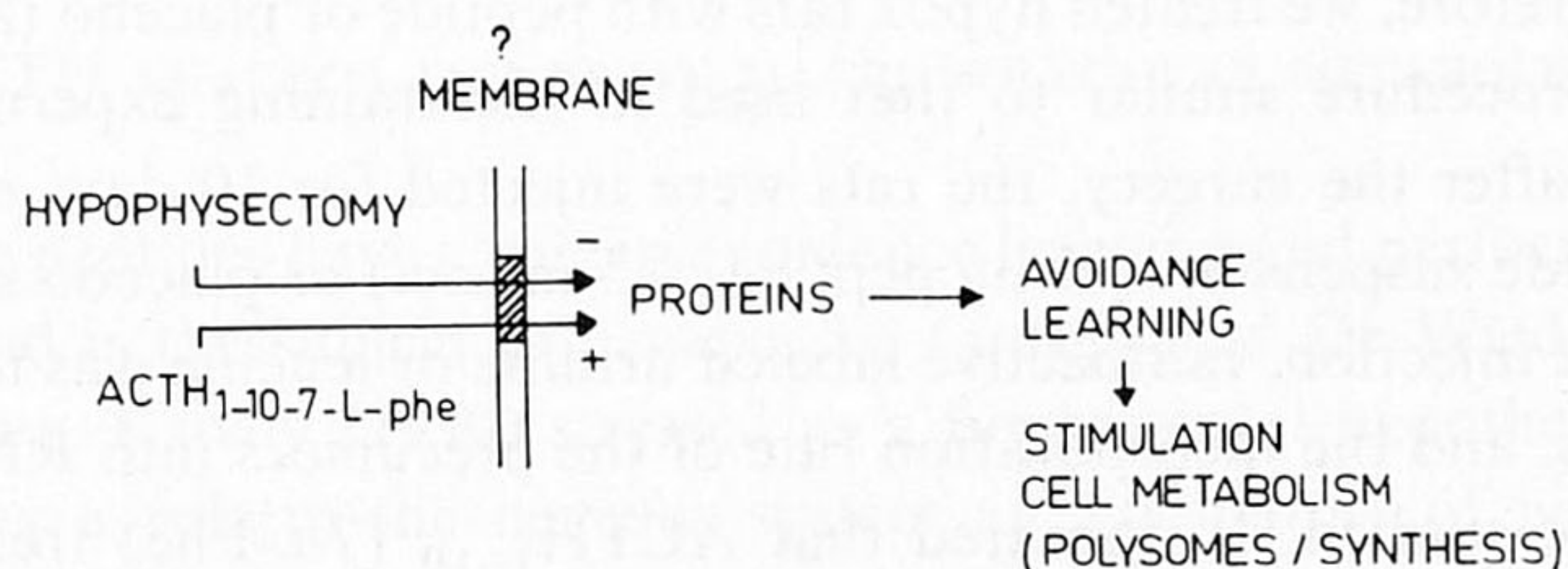


Fig. 1. Hypothetical model of the action of hypophysectomy and ACTH_{1-10} on rat brain and behavior.

Treatment of hypox rats with these peptides enhanced (ACTH_{1-10} -7-L-Phe), impaired (ACTH_{1-10} -7-L-Phe), or did not affect (ACTH_{11-24}) the incorporation of [^3H]-leucine into brain stem cytoplasmic proteins. The observation that hypophysectomy *per se* also affected this incorporation of leucine is further evidence that brain stem macromolecular metabolism and avoidance learning of rats are closely related to one another (assuming that the incorporation rate of leucine into proteins under the present conditions reflects synthesis of proteins). In Fig. 1, a tentative scheme is given, representing the current interpretation of our data.

Removal of the pituitary would deplete the rate of pituitary peptides and their breakdown products. These peptides would play a crucial role in certain avoidance learning of the animal. Their mechanism of action would be an interaction with the cell membranes of certain brain centers (adenylcyclase?) leading to an enhanced production of certain proteins which appear to be absent from the hypox rat. As a result of the lack of these pituitary peptides the hypox rat is unable to store the information necessary to master the task in long-term shuttle-box conditioning. Substitution of ACTH-like peptides enhances the production of the proteins involved and therefore leads to a normal acquisition behavior. This in turn would run parallel with a hyperactivity of the circuits involved in the acquisition process and this would account for the observed increase in brain stem polysomes. Therefore, acquisition would trigger an alteration in macromolecular metabolism; and it is likely that subsequent consolidation of the newly acquired information is dependent on this specific alteration. However, it should be kept in mind that the model is only speculative at present. More work is in progress to assess the validity of the hypothesis.

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DISCUSSION

CIARANELLO: Hypophysectomy markedly alters the content of catecholamine-synthesizing enzymes in the adrenal. However, in work done with Drs. Danellis and Deguchi, we were unable to find any effects of the hypophysectomy on the activity of tryptophan hydroxylase or tyrosine hydroxylase in the brain stem.

GISPEN: It has been shown by several authors that hypophysectomy reduces the turnover rate of neurotransmitters in brain (Fuxe and Hökfelt, *Progr. in Brain Res.*, Vol. 32, 42, 1970, Weiss *et al.*, *Amer. J. Physiol.*, 218, 864, 1970). Also Dr. Versteeg in our laboratory showed a decrease in turnover of noradrenaline, dopamine and serotonin in rat brain after hypophysectomy (Versteeg *et al.*, *Advanc. Biochem. Psychopharmacol.*, 6, 219, 1972). Maybe Dr. Hyypä could elaborate on this.

HYYPÄ: My data (Hyypä and Valavaara, 1970) are relevant to their content; really we found a marked increase in noradrenaline concentration of rat diencephalon. No changes were found in cerebral cortex. Hypophysectomy was performed in 3-week-old rats and amine analyses were made 2 months later.

SHASKAN: In studies in collaboration with Dr. Snyder at Johns Hopkins (*J. Neurochem.*, in press) we showed that the turnover of spermidine after intraventricular injection of [³H]putresine had a high turnover rate in brain stem areas (*i.e.* hypothalamus, midbrain, pons and medulla oblongata). Other areas showed markedly lower turnover rates. Since the turnover rate of spermidine has been shown to be closely related to synthesis of ribosomal RNA I feel that our results substantiate your findings.

GISPEN: Our data on uridine and leucine incorporation do indeed suggest that the rate of macromolecule metabolism is relatively high in brain stem areas as compared to the cortex.

WEISS: I am sure you are aware that there are far more differences between an animal that can avoid or escape a shock, and a yoked animal that is helpless than just a difference in learning. I wonder how you feel other differences influenced the data you spoke about and how adequate you feel this design is for studying biochemical changes in learning.

GISPEN: At present I think the quiet control is the best one to use in comparing a trained animal to another animal. For, as you also indicated, the yoked control apparently goes through quite a different type of stress and experience. Therefore, a yoked animal should not be used as the only control for training specific stimuli. On the other hand I would say that the fact that in our studies on phosphorylation of synaptosomal proteins (Glassman *et al.*, 1972) the trained mouse differed in this respect from the quiet animal and that no difference in phosphorylation between yoked and quiet could be detected, might imply that the experimental design used (*i.e.* short-term jump-box conditioning) is indeed adequate to pick up biochemical changes related to a training experience involving learning.