The Neuropeptides
Pharmacology Biochemistry and Behavior, Vol. 5, Suppl. 1, pp. 139–145. ANKHO International Inc., 1976.

Neurochemical Responses of Mice to ACTH and Lysine Vasopressin

ADRIAN J. DUNN, P. MICHAEL IUVONE AND HOWARD D. REES

Department of Neuroscience, University of Florida, College of Medicine
Gainesville FL 32610 U.S.A.

DUNN, A. J., P. M. IUVONE AND H. D. REES. *Neurochemical responses of mice to ACTH and lysine vasopressin.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 139–145, 1976. – Subcutaneous administration of ACTH 1-24 to mice increased the incorporation of [^{3}H]lysine into brain and liver proteins, an effect which resembled that due to footshock. Corticosterone administration did not mimic these effects. ACTH 4-10 increased the [^{3}H]lysine incorporation into brain but not liver protein. Lysine vasopressin (LVP) did not alter [^{3}H]lysine incorporation into brain or liver. These results are consistent with ACTH mediating the effects of footshock. However, dexamethasone decreased the brain responses to both footshock and ACTH, but while the liver response to ACTH was blocked, the footshock response was only diminished. This suggests a neural component in the response of the liver and possibly the brain. Intraventricular administration of ACTH 1-24 or ACTH 4-10 (D-phe), but not ACTH 4-10, increased [^{3}H]lysine incorporation into brain protein. These neurochemical responses parallelled a distinctive pattern of behavior characterized by stretching, yawning and excessive grooming. Treatment for 3 days with long-acting preparations of ACTH 4-10, ACTH 4-10 (D-phe) or ACTH 1-24 increased the conversion of [^{3}H]tyrosine into dopamine but not norepinephrine. α-MSH, β-MSH or LVP had no such effect. Similar treatment with ACTH 4-10 or ACTH 1-24 increased striatal tyrosine hydroxylase activity measured *in vitro*, but did not significantly alter the enzyme activity from other brain regions. We conclude that ACTH peptides can stimulate protein and dopamine metabolism in mouse brain and that LVP has no such effects.

ACTH 1-24 ACTH 4-10 Lysine vasopressin Protein synthesis Catecholamine synthesis
Tyrosine hydroxylase Stretching and yawning Grooming

IN VIEW of the widely reported behavioral effects of ACTH peptides and lysine vasopressin (LVP) [6] it is pertinent to ask what the mechanisms of action are. A good approach to this question is to determine the neurochemical consequences of administration of the peptides. This paper is concerned with three biochemical measures: [^{3}H]lysine incorporation into protein as a measure of protein synthesis, [^{3}H]tyrosine incorporation into catecholamines as a measure of catecholamine turnover, and a preliminary study on the activity of tyrosine hydroxylase, a key enzyme of catecholamine synthesis.

PROTEIN METABOLISM

Method

C57Bl/6J male mice were obtained from Jackson Laboratories (Bar Harbor, Maine). ACTH 1-24, ACTH 4-10 (L-phe) (OI63) and ACTH 4-10 (D-phe) (OI64) were obtained from Organon International, and lysine vasopressin from Sigma Chemical Co. L-[4,5-^{3}H]Lysine was obtained from Amersham Searle Inc.

Mice were kept in individual cages for 3 to 5 days before each experiment. Hormones were injected subcutaneously, or intraventricularly by the method of Dunn [7] but using methoxyflurane anesthesia. [^{3}H]Lysine was injected subcutaneously at a dose of 1 μCi/g and mice were sacrificed 10 min later. Analysis of radioactivity in free lysine and protein was performed as previously described [23]. The data on the incorporation of [^{3}H]lysine into protein are presented as the relative radioactivity (RR), equal to the protein radioactivity divided by the free lysine radioactivity. The RR thus corrects for variation in the uptake of precursor. (For a full analysis see ref. [8]).

Results

In Fig. 1 are presented the data on the incorporation of [^{3}H]lysine into brain and liver proteins. Compared to saline, subcutaneous ACTH 1-24 and ACTH 4-10 both significantly increased the incorporation into brain protein. In the liver ACTH 1-24, but not ACTH 4-10, increased the incorporation although saline injections alone produced a strong effect. LVP did not alter the incorporation in either tissue. At higher doses LVP inhibited the appearance of [^{3}H]lysine in the blood and in the brain and liver [8]. It can be seen that ACTH 1-24 mimics the effect of footshock in both brain and liver. Thus, the incorporation changes in response to footshock or saline might be accounted for by secretion of pituitary ACTH in response to these stressful procedures. Adrenal corticosterone is apparently not involved since the responses to footshock were still present in adrenalectomized mice, and corticosterone administration did not change the [^{3}H]lysine incorporation.

A further study was performed in mice pretreated with dexamethasone, a potent glucocorticoid which suppresses ACTH release. Dexamethasone itself did not change [^{3}H]lysine incorporation into mouse brain, but approximately doubled the uptake of the amino acid into the liver without any change in the incorporation into protein [21]. In the brain the biochemical responses to footshock,

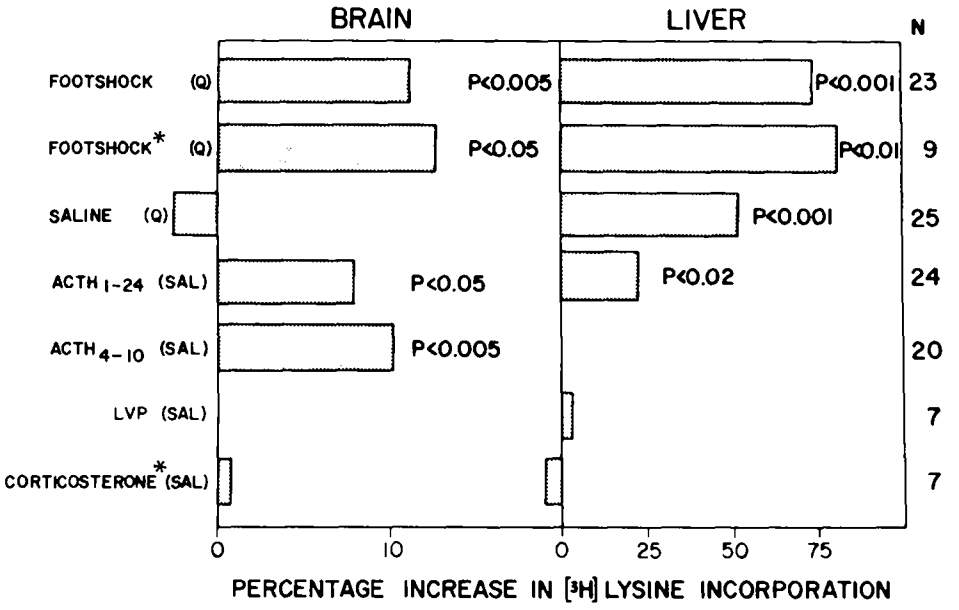

FIG. 1. Effect of footshock or hormones on the incorporation of [^{3}H]lysine into mouse brain and liver protein. C57B1/6J male mice were treated with 20 footshocks (0.3 mA, 1 sec) in 15 min and injected with [^{3}H]lysine 20 min later. Hormones were injected subcutaneously at the following doses per g body weight: ACTH 1-24 (0.6 μg), ACTH 4-10 (0.3 μg), LVP (4 ng), corticosterone (5 μg), followed 15 min later by [^{3}H]lysine. Mice were sacrificed 10 min after [^{3}H]lysine injection. All results are the relative radioactivity (protein radioactivity/free lysine radioactivity) expressed as a percentage increase over control (Q = quiet, SAL = saline-injected). Statistics are calculated by Student's *t*-test, or Dunnett's test where more than one group was involved. *Mice were adrenalectomized 3 days before the experiment. For full details see refs. [8] and [21] from which these data were compiled.

ACTH 1-24 and ACTH 4-10 were diminished; the small increases in [^{3}H]lysine incorporation were not statistically significant (Fig. 2). In the liver dexamethasone only partly prevented the response to footshock, but the response to ACTH 1-24 was completely blocked. These data suggest the brain response might be mediated by ACTH, but that ACTH is not necessary for the liver response. Perhaps there is sympathetic control in the liver since sympathetic stimulation has been shown to induce hepatic enzymes [3]. The data further suggest that dexamethasone directly antagonizes the liver response, and possibly also the brain response.

The administration of exogenous ACTH generally produced smaller and less consistent responses than footshock. This might indicate that a neural influence was also involved in the cerebral response, but it may also indicate

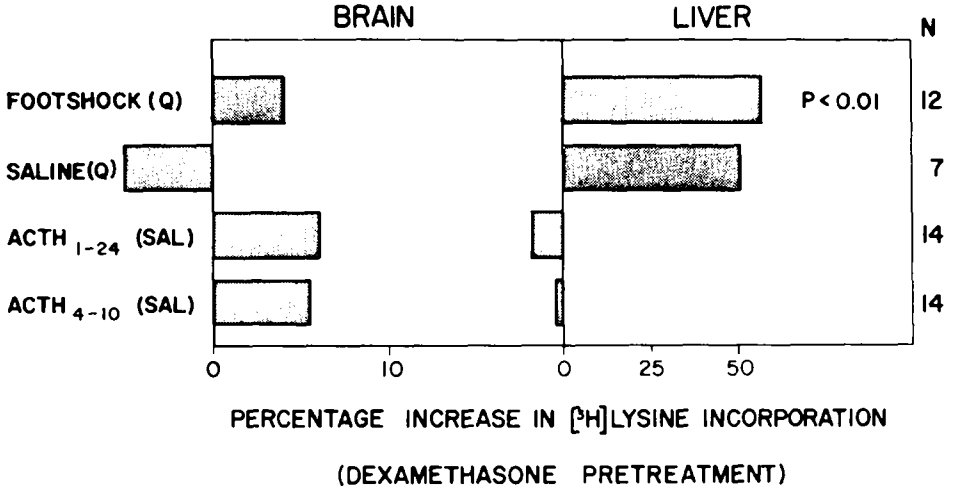

FIG. 2. Effect of hormones on [^{3}H] lysine incorporation into brain and liver proteins of mice pretreated with dexamethasone. Details as for Fig. 1 except that mice were pretreated 3 hr before hormone injection with 300 ng/g dexamethasone. Data are condensed from refs. [9] and [21].

that the administered ACTH was entering the brain only to a very limited extent. There is now evidence that ACTH may enter the cerebrospinal fluid directly from the pituitary rather than via the peripheral circulation [1]. Therefore we administered ACTH peptides directly into the lateral cerebral ventricles and examined the [^{3}H]lysine incorporation. Figure 3 shows that both ACTH 1-24 and ACTH 4-10 (D-phe) increased the [^{3}H]lysine incorporation into brain protein: ACTH 4-10 produced a smaller nonsignificant effect. These results strongly indicate that the effect of ACTH on [^{3}H]lysine incorporation is central, although we are unable to explain the ineffectiveness of ACTH 4-10. Dexamethasone pretreatment suppressed the biochemical responses, again suggesting direct antagonism (Rees and Dunn, unpublished observations). Since the intraventricular administration of ACTH peptides has been reported to result in various characteristic behaviors [10,12], we also studied these in the same animals. Table 1 shows that both ACTH 1-24 and ACTH 4-10 (D-phe) but not the other peptides enhanced stretching and yawning behavior. These same 2 peptides also increased the proportion of time mice spent grooming (Fig. 4) in agreement with the results of Gispen *et al.* [12] in rats. Thus there was a good correlation between behavioral activity and the [^{3}H]lysine incorporation changes. Dexamethasone pretreatment did not abolish the behavioral responses to ACTH 1-24 and ACTH 4-10 (D-phe) although the responses may have been depressed (Rees and Dunn, unpublished observations). This might indicate that the behavioral and biochemical responses are differentially mediated, or that the dexamethasone antagonized the [^{3}H]lysine incorporation change at a later step.

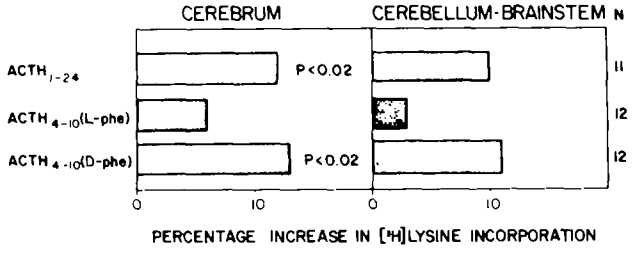

FIG. 3. Effect of intraventricular hormones on [^{3}H]lysine incorporation into brain protein. Hormones (2.5 nmoles total) or placebo (an amino acid mixture corresponding to the components of ACTH 4-10) were injected bilaterally (5 μl each side) into the lateral ventricles of C57Bl/6J mice 75 min before [^{3}H]lysine injection. Biochemical analysis as in Figure 1 except that the brain was divided into two parts by a midcollicular section. The data are from ref. [23].

LVP caused a dramatic hyperactivity in the mice, which were never still and constantly foraging, exploring, rearing or grooming. Some of them exhibited prolonged squeaking. We have also observed this behavior in CD-1 mice but not in rats. This behavior was not accompanied by any changes in [^{3}H]lysine incorporation in CD-1 mice [22].

Discussion

Our results indicating that ACTH increased the incorporation of amino acids into brain protein are consistent with the data of others in mice [27,29] and rats [15, 20, 28]. That the effect is a direct one on the brain is indicated by our results from the intraventricular injections and by the observation that ACTH 1-10 in vitro stimulates the incorporation of amino acids into proteins in caudal thalamic brain slices from hypophysectomized rats [24]. With incorporation changes of such small magnitude, it is difficult to prove unequivocally that a change in the rate of protein synthesis has occurred. Nevertheless, Schotman *et al.* [28] showed that ACTH 1-10 treatment of hypophysectomized rats that were trained in shuttle-box avoidance increased the aggregation of ribosomes into polyribosomes, strongly indicative of an increased rate of protein synthesis. However, Reith *et al.* [25] found that the increase of amino acid incorporation induced by ACTH 1-10 in hypophysectomized rats was not selective for any particular protein species separated by polyacrylamide gel electrophoresis.

To relate these apparent changes in brain protein synthesis to the behavioral changes induced by ACTH peptides may be premature. It is pertinent that protein synthesis appears to be necessary for the consolidation of memory [4], that many amnestic agents cause protein synthesis inhibition [9] and that ACTH peptides can reverse amnesia due to CO_2 [26], ECS [17] or anisomycin (Dunn, unpublished observations). However, LVP, which is a more potent amnesia-reversing agent than ACTH [26], did not change the protein synthesis rate. Thus the correlation between cerebral protein synthesis and learning and memory enhancement may not be causal.

TABLE 1

STRETCHING AND YAWNING BEHAVIOR FOLLOWING INTRAVENTRICULAR ACTH

Injectate	N	Stretching	Yawning	Stretching-Yawning
Placebo	16	2	0	0
$ACTH_{1-24}$	12	10†	9†	9†
$ACTH_{4-10}$ (L-phe)	12	4	1	0
$ACTH_{4-10}$ (D-phe)	12	7*	4*	4*

Number of mice exhibiting the specified behavior in an 85 min period following bilateral intraventricular administration of peptides or an amino acid mixture in the experiment of Fig. 3. Stretching-yawning refers to synchronized stretching and yawning behavior. Data are from [22].

*Significantly different from Placebo (Fisher exact probability test), $p<0.05$.

†$p<0.001$.

CATECHOLAMINES

Since in many respects the behavioral actions of ACTH resemble those of adrenergic stimulants, we investigated catecholamine (CA) metabolism in mice. In previous studies Versteeg *et al.* [31,32] had shown that the rate of depletion of cerebral norepinephrine (NE) in rats treated with α-methyl-p-tyrosine (AMPT) was increased by ACTH 4-10 but not by ACTH 4-10 (D-phe). This suggested that ACTH 4-10 increased the turnover of cerebral NE.

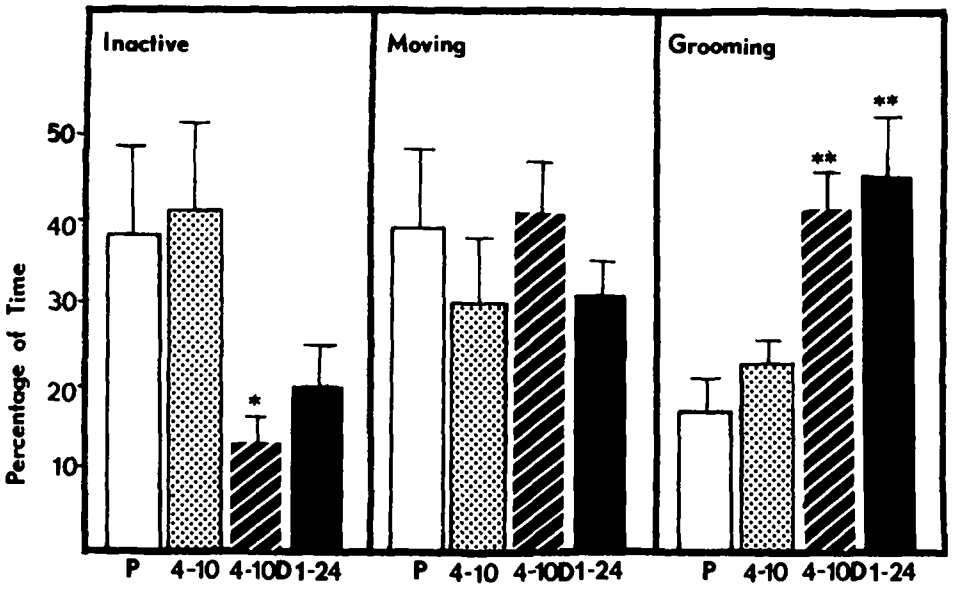

FIG. 4. Behavioral observations on mice injected intraventricularly with ACTH peptides. Behavioral observations were recorded every 2.5 min in the same experiment as Fig. 3. The data are from ref. [23].

Using a similar technique Leonard [19] found that NE turnover was increased by either ACTH 4-10 or ACTH 4-10 (D-phe), the latter in disagreement with the results of Versteeg [31]. Leonard also found that ACTH 4-10 increased the turnover of dopamine (DA) in the midbrain (striatum, hippocampus, thalamus, hypothalamus and amygdaloid cortex) of the rat. The AMPT method for measuring CA turnover has been criticized as nonphysiological, and results conflicting with tracer techniques for measuring catecholamine metabolism have been obtained [3]. Subsequently, Versteeg and Wurtman [33] found that ACTH 4-10 increased the conversion of [^{3}H]tyrosine into total cerebral catecholamines. Both adrenalectomy and hypophysectomy blocked the ACTH 4-10-induced effects on CA metabolism [32,33].

Glucocorticoids also appear to affect catecholamine metabolism. Adrenalectomy increased the turnover of cerebral NE [11,16], while subsequent replacement therapy partially blocked the increased NE turnover [16]. This suggests that glucocorticoids decrease cerebral NE metabolism.

Method

Male CD-1 mice (Charles River) were treated daily for 3 days with a long-acting zinc phosphate-peptide suspension or with the zinc phosphate vehicle. All peptides were administered at a dose of 3.4×10^{-10} moles/g. Corticosterone (15 μg/g) was administered suspended in saline. Approximately 24 hr after the last peptide administration or 60 min after a single corticosterone (or saline control) injection the mice were injected with 5 μCi/g of [2,6-^{3}H]tyrosine (Amersham-Searle, Inc.) and sacrificed by decapitation 10 min later. The concentrations and specific radioactivities of tyrosine, DA and NE were determined after separation by cation-exchange and alumina chromatography (see ref. [14] for full details). The specific radioactivity of protein was determined after trichloroacetic acid precipitation [13]. The labeling of NE, DA and protein were corrected for uptake of the precursor by dividing the specific activity of the product by the specific activity of tyrosine, to obtain a relative specific activity (RSA).

Tyrosine hydroxylase (TH) was assayed in mouse brain regions using a similar protocol. Twenty-four hr after the third daily peptide injection the mice were killed by decapitation and the brains rapidly removed and dissected on ice into brain stem (mesencephalon and metencephalon minus cerebellum), hippocampus, hypothalamus, septal area and striatum. The brain parts were frozen in liquid N_2 and stored at −50° C until assayed.

The frozen brains were weighed and homogenized in 1:8 volumes of phosphate buffer (0.1 M potassium phosphate, 100 μM $CaCl_2$, 5 μM $FeCl_2$, 0.5% Triton X-100, pH 6.0) with a motor driven glass-teflon homogenizer. The homogenates were centrifuged for 30 min at 45,000 × g. Ten μl aliquots of this supernatant were incubated at 37° C for 40 min with 10 μl of incubation buffer containing 0.1 M potassium phosphate, 100 μM $CaCl_2$, 5 μM $FeCl_2$, 2.56 mM tetrahydrobiopterin (Hoffman-La Roche, Inc.), 280 mM 2-mercaptoethanol, 200 μM L-tyrosine, 4.0 U/μl catalase and 0.065 μCi/μl [2,6-^{3}H]tyrosine. The reaction was stopped by addition of 200 μl of 5% trichloroacetic acid, and 200 μl of 0.1 M tris (hydroxymethyl)aminomethane and NaOH were added to raise the pH to 7.8–8.6. The samples were then applied to 6 mm dia. columns containing 125 mg of Al_2O_3 (prepared according to ref. [2]) and equilibrated at pH 8.4 with 0.1 M tris. The columns were washed with 20 ml of distilled H_2O and the reaction products eluted into scintillation vials with 2.0 ml of 1.0 N HCl. To the vials, 15 ml of Triton-toluene scintillator (8 g/l PPO, 0.1 g/l POPOP in toluene mixed 2:1 (v/v) with Triton X-100) was added. Boiled enzyme blanks, taken from each brain region, were used to control for auto-oxidation of tyrosine.

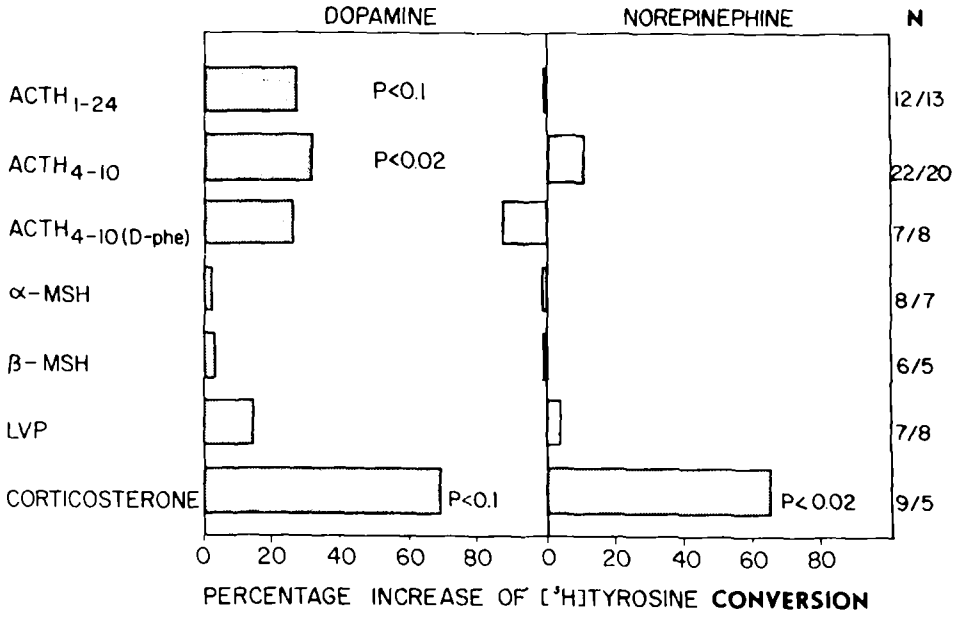

FIG. 5. Effect of hormonal treatment on the conversion of [^{3}H]tyrosine to dopamine and norepinephrine in the brains of CD-1 mice. Data are the relative specific activity (dopamine or norepinephrine specific activity/tyrosine specific activity) as a percentage of control and are compiled from ref. [13] and [14].

Results

Treatment with ACTH 1-24 or ACTH 4-10 increased the specific activity and RSA of dopamine (Fig. 5). Since neither peptide influenced the cerebral content of DA this suggests that ACTH 1-24 or ACTH 4-10 treatment increased the rate of DA turnover in mouse brain. These peptides had no detectable effect on the specific activity or relative specific activity of NE, but ACTH 1-24 significantly reduced the whole brain content by 11% [14].

ACTH 4-10 (D-phe) increased the RSA of DA and decreased that of NE (Fig. 5) but these effects were not significant probably due to the large variance and small sample size. ACTH 4-10 (D-phe) had no effect on the cerebral cerebral contents of NE or DA. α-MSH, β-MSH or LVP caused no change in either the endogenous content nor the RSA of NE and DA.

Acute corticosterone administration increased the relative specific activity of both NE and DA (Fig. 5) but had no effect on the cerebral content of either amine. This indicates that corticosterone administration to intact mice resulted in an increase in catecholamine turnover.

In these experiments the incorporation of [^{3}H]tyrosine into protein was also studied. None of the hormonal treatments altered this incorporation [14]. This result does not conflict with those reported above since a different precursor was used, and the mode of ACTH administration was different. However, the mouse strain difference may also be important. As opposed to the results obtained in C57B1/6J mice, footshock caused only small nonsignificnat increases in the incorporation of [^{3}H]lysine into brain proteins in CD-1 or Swiss/ICR mice. However, the liver responses resembled those in C58B1/6J mice. Likewise ACTH 1-24 either peripherally or intraventricularly did not significantly increase the [^{3}H]lysine incorporation into brain proteins of CD-1 mice, although again liver responses were observed. Following intraventricular injections the behavioral responses of CD-1 mice to ACTH 1-24, ACTH 4-10, ACTH 4-10 (D-phe) and LVP were similar to those observed in C57B1/6J mice. The significance of these strain differences is not known, but Rudman *et al.* [27] observed ACTH-induced increases in amino acid incorporation in CF-1 mice and Semiginovsky and Jakoubek [29] observed changes in A_2 mice.

In a preliminary study we assayed the activity of tyrosine hydroxylase (TH) in ACTH 1-24- and ACTH 4-10-treated mice (Fig. 6). Both ACTH 1-24 and ACTH 4-10 significantly increased TH activity in the striatum, a result which is consistent with our observed increase in whole brain dopamine turnover. The only other brain region which appeared to be altered was the septum, where ACTH treatment produced nonsignificant decreases in TH activity.

Discussion

Our results indicate that ACTH 4-10 and ACTH 1-24 increase the turnover of cerebral DA and the activity of striatal tyrosine hydroxylase. These effects of ACTH analogs in CD-1 mice are in general agreement with the previously reported effects in the rat, however, there are important differences. Both Versteeg [31] and Leonard [19] observed increases in NE depletion after AMPT in ACTH 4-10-treated rats. But, Leonard found that the ACTH 4-10 effect on DA depletion was more than twice as large as the effect of NE depletion, suggesting that even in the rat the major effect of ACTH 4-10 is on DA metabolism.

Although ACTH 1-24 and ACTH 4-10 increased the turnover of DA in mouse brain, α-MSH and β-MSH were ineffective. This is difficult to explain since the ACTH 4-10 sequence is contained in both MSH's. Nevertheless the results are in agreement with those of Kostrzewa *et al.* [18] who reported that α-MSH had no effect on the AMPT-induced depletion of striatal DA in endocrinologically intact rats. Thus apparently ACTH 4-10, but not α-MSH,

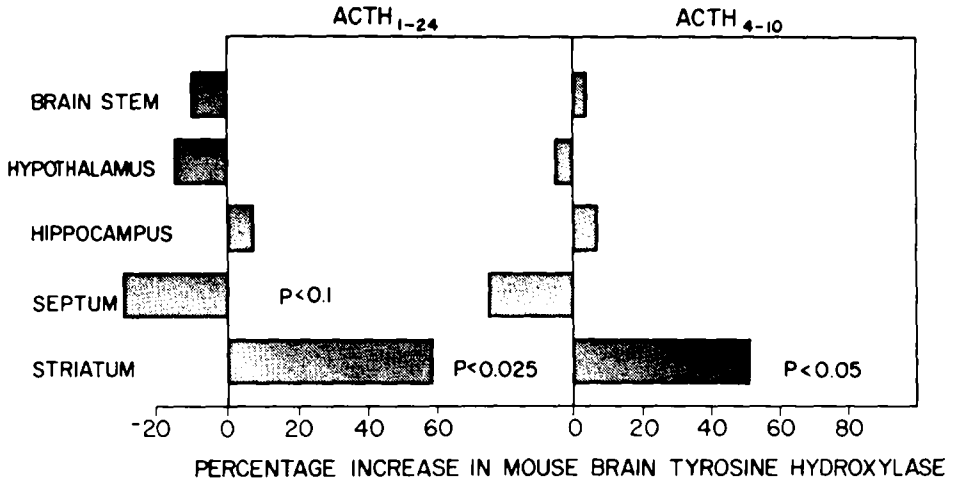

FIG. 6. Effect of ACTH 1-24- or ACTH 4-10- treatment on tyrosine hydroxylase activity of mouse brain regions. Data are presented as percentage increase over control. Statistics by Dunnett's test.

mimics the action of ACTH with respect to DA metabolism.

Corticosterone increased the turnover of both NE and DA. Although the dose used in the present experiment was large, we have previously found that corticosterone increases NE turnover in a dose-dependent manner [13]. Many recent data suggest that to determine physiological effects of pituitary adrenal hormones on catecholamine metabolism, one must inject the hormones into endocrinologically intact animals (for discussion see refs. [13,14]). Thus, the effects of corticosteroid administration on NE turnover are opposite in intact [13] and adrenalectomized [11] rodents.

There have been no previous reports on tyrosine hydroxylase activity following ACTH treatment, but Van Loon and Mascardo [30] showed that ACTH 1-24 or ACTH 1-10 increased the activity of dopamine-β-hydroxylase in the brain stem and hypothalamus of hypophysectomized rats. It is not possible to reconcile these results without further data.

GENERAL DISCUSSION

To attempt to relate the neurochemical effects of the peptides to their reported behavioral effects may be premature. Nevertheless, some conclusions can be drawn. Since we did not detect neurochemical effects to LVP we know of no correlates of its behavioral activity. Insofar as ACTH peptides did cause neurochemical changes, we may say that if LVP and ACTH act similarly on behavior then our chemical changes do not correlate. However, the behavioral activities of LVP and ACTH may be distinct [6].

Of most importance are the results with ACTH 4-10 containing the D- and L-analogs of phenylalanine. The results on protein synthesis are puzzling, since administered peripherally, ACTH 4-10 mimicked ACTH 1-24, yet centrally ACTH 4-10 (D-phe) but not ACTH 4-10 acted like ACTH 1-24. These data are very hard to relate to those on avoidance behavior. The effects on dopamine metabolism are easier to relate. If we accept that ACTH 4-10 (D-phe) increases DA turnover, then all the ACTH analogs had a similar effect. This is inconsistent with the opposite effects of ACTH 4-10 and ACTH 4-10 (D-phe) on the extinction of active avoidance behavior, but consistent with their similar effects on passive avoidance [6]. Effects on extinction are hard to interpret, but the mediation of ACTH-induced behaviors by dopaminergic systems is consistent with the known functions of such systems.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institute of Mental Health (MH25486) and the Alfred P. Sloan Foundation. HDR was supported by a postdoctoral fellowship from NINCHDS (N502469). PMI was supported by a graduate training grant from NIMH (MY10320). The technical assistance of Byron Bergert, Fran Bryan, Bonnie Collier, Dick Delanoy, Michael Kearin and Joan Morasco is gratefully appreciated. ACTH 1-24, ACTH 4-10 (0163) and ACTH 4-10 (D-phe) (0164) were generous gifts from Dr. Henk van Riezen, Organon International, Oss, The Netherlands. α-MSH and β-MSH were generous gifts from Dr. Saul Lande, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut. Tetrahydrobiopterin dihydrochloride was a generous gift from Dr. W. E. Scott, Hoffman-La Roche, New York.

REFERENCES

1. Allen, J. P., J. W. Kendall, R. McGilvra and C. Vancura. Immunoreactive ACTH in cerebrospinal fluid. *J. clin. Endocr. Metab.* 38: 586–593, 1974.
2. Anton, A. J. and D. F. Sayre. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac.* 138: 360–375, 1962.
3. Anton-Tay, F., S. M. Anton and R. J. Wurtman. Mechanisms of changes in brain norepinephrine metabolism after ovariectomy. *Neuroendocrinology* 6: 265–273, 1970.
4. Barondes, S. H. Cerebral protein synthesis inhibitors block long-term memory. *Int. Rev. Neurobiol.* 12: 177–205, 1970.
5. Black, I. B. and D. J. Reis. Central neural regulation by adrenergic nerves of the daily rhythm in hepatic tyrosine transaminase activity. *J. Physiol.* 219: 267–280, 1971.
6. de Wied, D. Pituitary-adrenal system hormones and behavior. In: *The Neurosciences Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge, Mass.: M.I.T. Press, 1974, pp. 653–666.

7. Dunn, A. J. Intracerebral injections inhibit amino acid incorporation into brain protein. *Brain Res.* **99:** 405–409, 1975.
8. Dunn, A. J., H. D. Rees and P. M. Iuvone. The role of pituitary hormones in the stimulation-induced changes in amino acid incorporation into mouse brain and liver protein: effects of exogenous $ACTH_{1-24}$, $ACTH_{4-10}$ and lysine vasopressin. *Neurochem. Res.* (submitted)
9. Dunn, A. J., A. Giuditta, J. F. Wilson and E. Glassman. The effect of electroshock on brain RNA and protein synthesis and its possible relationship to behavioral effects. In: *The Psychobiology of Convulsive Therapy*, edited by M. Fink, J. McGaugh, S. Kety and T. A. Williams. Washington, D.C.: V. H. Winston and Sons, 1974, pp. 185–198.
10. Ferrari, W., G. L. Gessa and L. Vargui. Behavioral effects induced by intracisternally injected ACTH and MSH. *Ann. N.Y. Acad. Sci.* **104:** 330–345, 1963.
11. Fuxe, K., H. Corrodi, R. Hokfelt and G. Jonsson. Central monoamine neurons and pituitary-adrenal activity. *Prog. Brain Res.* **32:** 42–56, 1970.
12. Gispen, W. H., V. M. Wiegant, H. M. Greven and D. de Wied. The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure-activity studies. *Life Sci.* **17:** 645–652, 1975.
13. Iuvone, P. M., J. Morasco and A. J. Dunn. Effect of corticosterone on the synthesis of catecholamines in the brains of CD-1 mice. *Brain Res.* (submitted)
14. Iuvone, P. M., R. L. Delanoy, J. Morasco and A. J. Dunn. Pituitary hormones and the synthesis of catecholamines in mouse brain. (in preparation).
15. Jakoubek, B., B. Semiginovsky and A. Dedicova. The influence of stress and ACTH on the protein synthesis in brain. *Activitas nerv. sup.* **13:** 140, 1971.
16. Javoy, F., J. Glowinski and C. Krodon. Effects of adrenalectomy on the turnover of norepinephrine in the rat brain. *Eur. J. Pharmac.* **4:** 103–104, 1968.
17. Keyes, J. B. Effect of ACTH on ECS-produced amnesia of a passive avoidance task. *Physiol. Psychol.* **2:** 307–309, 1974.
18. Kostrzewa, F., A. Kastin and M. Spirtes. α-MSH and MIF-I effects on catecholamine levels and synthesis in various rat brain areas. *Pharmac. Biochem. Behav.* **3:** 1017–1023, 1975.
19. Leonard, B. E. The effect of two synthetic ACTH analogues on the metabolism of biogenic amines in the rat brain. *Arch. int. Pharmacodyn. Ther.* **207:** 242–253, 1974.
20. Reading, H. W. Effects of some adrenocorticotrophin analogues on protein synthesis in brain. *Biochem. J.* **127:** 7P, 1972.
21. Rees, H. D. and A. J. Dunn. The role of pituitary-adrenal system in the footshock-induced increase of [^{3}H]lysine incorporation into mouse brain and liver proteins. *Brain Res.*, 1976, (in press)
22. Rees, H. D., A. J. Dunn and P. M. Iuvone. Behavioral and biochemical responses of mice to the intraventricular administration of ACTH analogs and lysine vasopressin. *Life Sci.* **18:** 1333–1340, 1976.
23. Rees, H. D., L. L. Brogan, D. J. Entingh, A. J. Dunn, P. G. Shinkman, T. Damstra-Entingh, J. F. Wilson and E. Glassman. Effect of sensory stimulation on the uptake and incorporation of radioactive lysine into protein of mouse brain and liver. *Brain Res.* **68:** 143–156, 1974.
24. Reith, M. E. A., P. Schotman and W. H. Gispen. Hypophysectomy, $ACTH_{1-10}$ and *in vitro* protein synthesis in rat brain stem slices. *Brain Res.* **81:** 571–575, 1974.
25. Reith, M. E. A., P. Schotman and W. H. Gispen. Incorporation of [^{3}H]leucine into brain stem protein fractions: the effect of a behaviorally active, N-terminal fragment of ACTH in hypophysectomized rats. *Neurobiology* **5:** 355–368, 1975.
26. Rigter, H., R. Elbertse and H. van Riezen. Time-dependent anti-amnesic effect of $ACTH_{4-10}$ and desglycinamide-lysine vasopressin. *Prog. Brain Res.* **42:** 164–171, 1975.
27. Rudman, D., S. W. Scott, A. E. Del Rio, D. H. Houser and S. Sheen. Effect of melanotrophic peptides on protein synthesis in mouse brain. *Am. J. Physiol.* **226:** 687–696, 1974.
28. Schotman, P., W. H. Gispen, W. S. Janz and D. de Wied. Effects of ACTH analogues on macromolecule metabolism in the brain and spinal cord of mice. *Brain Res.* **46:** 347–362, 1972.
29. Semiginovsky, B. and B. Jakoubek. Effects of ACTH on the incorporation of L-[U-^{14}C] leucine into the brain and spinal cord of mice. *Brain Res.* **35:** 319–323, 1971.
30. Van Loon, G. R. and R. N. Mascardo. Effect of hypophysectomy, ACTH and dexamethasone on brain dopamine beta hydroxylase activity in rats. Proc. 5th A. *Meeting Soc. Neurosci.* 1975, p. 444.
31. Versteeg, D. Effect of two ACTH analogs on noradrenaline metabolism in rat brain. *Brain Res.* **49:** 483–485, 1973.
32. Versteeg, D. H. G. and R. Wurtman. Effects of $ACTH_{4-10}$ on the rate of synthesis of [^{3}H]catecholamines in the brains of intact, hypophysectomized and adrenalectomized rats. *Brain Res.* **93:** 552–557, 1975.
33. Versteeg, D. H. G., W. H. Gispen, P. Schotman, A. Witter and D. deWied. Hypophysectomy and rat brain metabolism: effects of synthetic ACTH analogs. *Adv. Biochem. Psychopharmac.* **6:** 219–239, 1972.