Pharmacology Biochemistry & Behavior, Vol. 9, pp. 21–26. Printed in the U.S.A.

Mouse Brain Deoxyglucose Uptake After Footshock, ACTH Analogs, α–MSH, Corticosterone or Lysine Vasopressin

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(Received 3 January 1978)

DELANOY, R. L. AND A. J. DUNN. *Mouse brain deoxyglucose uptake after footshock, ACTH analogs, α–MSH, corticosterone or lysine vasopressin.* PHARMAC. BIOCHEM. BEHAV. 9(1) 21–26, 1978.—The cerebral uptake of subcutaneously injected [^{3}H]2-deoxy-D-glucose (2DG) in 16 brain regions was examined following 30 noncontingent random footshocks or the acute injection of saline, $ACTH_{1-24}$ (0.5 μg/g), $ACTH/MSH_{4-10}$ (0.25 μg/g), [D-Phe_7]$ACTH_{4-10}$ (0.25 μg/g), [Met_4SO_2,D-Lys_8,Phe_9]$ACTH_{4-9}$ (0.01 μg/g), α–MSH (0.5 μg/g), corticosterone (2.5 μg/g) or lysine vasopressin (0.05 μg/g). Footshock selectively decreased 2DG uptake in parietal cortex and brain stem, and increased that in the hypothalamus. Whole brain 2DG uptake was decreased by injection of saline or most of the hormones relative to uninjected animals, but this effect was probably peripheral since plasma glucose content was increased by the injections. The only regionally specific effect of the hormones was an increased 2DG uptake in olfactory bulb by saline, $ACTH/MSH_{4-10}$ and corticosterone relative to uninjected animals. Since α–MSH had been reported previously to decrease blood flow (measured by antipyrene uptake) in all brain regions except occipital cortex [5,6], we directly compared antipyrene uptake with 2DG uptake in the same animals using a double-isotope procedure. The results revealed an increase in 2DG uptake relative to antipyrene in cortical regions relative to subcortical regions, contradicting earlier assumptions [19].

2-Deoxyglucose Blood flow Antipyrene Footshock Mouse brain ACTH Vasopressin α–MSH Corticosterone

MANY reports in the last decade have indicated that the pituitary peptides, adrenocorticotrophin (ACTH), α-melanocyte-stimulating hormone (MSH), and lysine vasopressin (LVP), alter the performance of animals in several learning tasks [11, 26, 27]. The mechanism by which these hormones alter performance is not understood, although it is clear that they are not essential for learning. DeWied [26] has suggested that ACTH and its analogs increase motivation. However, based on improved performance in tasks designed to measure attention in both humans and rodents, Kastin and his colleagues [11] favor an effect of MSH or ACTH analogs on selective attention.

The effects of these hormones are not particularly specific behaviorally, and it is likely that the effects on brain metabolism are widespread. Indeed, many effects of ACTH, MSH and their analogs on cerebral metabolism have been reported [3]. Such effects might well be mediated through one or other of the catecholaminergic systems, especially since ACTH and its analogs [10, 14, 25], α–MSH [12,15], LVP [23], and corticosterone [9], have all been reported to increase catecholamine turnover in rodents. The catecholaminergic systems are widely distributed throughout the brain and have also been implicated in learning and memory [8]. Furthermore, there is extensive noradrenergic innervation of cerebral capillaries which may regulate cerebral blood flow [17]. It has also been shown that lesions of ascending dopaminergic fibers resulted in decreased forebrain glucose uptake [20].

Goldman *et al.* [5,6] reported that injections of α–MSH at a dose that improved performance in behavioral tasks, decreased the antipyrene uptake in all rat brain regions tested, except visual cortex which maintained a constant rate of uptake. They suggested that this regionally specific effect reflected the improved performance observed in visual tasks, and the concurrent lack of effect in auditory discrimination tasks.

Recently, Sokoloff and his colleagues have described a procedure for the measurement of cerebral glucose uptake on a regional basis [22]. The technique uses 2-deoxyglucose (2DG), an analog of glucose which is taken up by brain cells at a rate proportional to glucose intake. It is then phosphorylated just as glucose is, but 2DG-6-phosphate is relatively stable metabolically and is sequestered inside the cell for many hours [22]. Brain 2DG uptake has been shown to be altered by a number of experimental manipulations [16,21].

Most previous studies have used [^{14}C]2-deoxyglucose and have assayed autoradiographically [16]. This technique is appropriate for the gross manipulations that have been reported so far, but autoradiography is difficult to quantitate, especially without elaborate equipment, and very little quantitative data have been reported. For the more subtle changes we anticipated following hormone treatments, we needed a procedure more readily quantifiable. Thus we chose to analyze the ^{3}H content in brain regions by scintillation counting of hand-dissected brain regions. This procedure has been observed to produce results qualitatively simi-

lar to those obtained by autoradiography. For example, decreases of uptake in visual system structures have been observed following enucleation of the chick (Gray, Iuvone and Dunn, unpublished observation). Our results show that this technique is sensitive to small changes in large regions, and enables large numbers of animals to be run in the same experiment. However, anatomical resolution is lost, so that very localized changes are not detected.

We first report the effects of footshock treatment on regional 2DG uptake. Then we present data on the regional 2DG uptake following injection of a number of hormones to answer two questions. To what extent are the effects of footshock treatment mimicked by the release of hormones? And, are there any regional effects of the hormones on cerebral glucose metabolism that might explain the behavioral effects, or help us to locate the site(s) of cerebral action of these hormones?

METHOD AND MATERIALS

Male CD–1 mice weighing 25–30 g at the time of the experiment were obtained from Charles River Laboratories. Animals were housed in groups of six or seven until 3 days prior to sacrifice when they were separated into individual cages.

For the footshock experiments, 30 noncontingent randomly timed 300 μA footshocks were administered during a 15 min period in the apparatus previously described [18]. Ten μCi of [^{3}H]2-deoxyglucose (Amersham-Searle, Inc., 19–20 Ci/mmole) was injected subcutaneouly immediately prior to footshock. Mice were returned to their home cages immediately following injection (Quiet group) or following the footshock period (Footshock group). The mice were sacrificed 45 min after the injection.

Two experiments were also performed following hormone injections. All animals were injected daily for 14 days with 0.1 ml of saline in order to habituate animals to handling and injection. $ACTH_{4-10}$, $ACTH_{1-24}$, [D-Phe_7]$ACTH_{4-10}$, [Met_4SO_2,D-Lys_8,Phe_9]$ACTH_{4-9}$ and α–MSH were provided by Dr. Henk Greven and Dr. Henk Van Riezen of Organon International B.V. Note that α–MSH is identical with [N-Acetyl Ser_1]$ACTH_{1-13}$ so that $ACTH_{4-10}$ can also be regarded as MSH_{4-10}. Corticosterone and LVP were obtained from Sigma Chemical Co. In the first experiment, [^{3}H]2DG uptake was measured following subcutaneous injections of saline, $ACTH_{4-10}$ (0.25 μg/g), $ACTH_{1-24}$ (0.5 μg/g), LVP (0.05 μg/g), corticosterone (2.5 μg/g) or no injection (Quiet). In the second experiment, the hormones included $ACTH_{4-10}$ and $ACTH_{1-24}$, as well as [D-Phe_7]$ACTH_{4-10}$ (0.25 μg/g), α–MSH (0.5 μg/g), [Met_1 SO_2, D-Lys_8, Phe_9] $ACTH_{4-9}$ (0.01 μg/g), and Saline and Quiet controls. The doses used correspond to those previously reported to affect avoidance learning [27]. Hormones were dissolved in isotonic saline containing 0.001 M HCl on the day of the experiment, except that when the experiment lasted 2 days, the same solution (stored frozen overnight) was used on the second day. Ten min following the hormone injection, 10 μCi of [^{3}H[2DG were injected subcutaneously. Animals were returned to their home cages and were decapitated 45 min later.

One experiment was designed to compare regional uptake distributions of [^{3}H]2DG and [^{14}C]antipyrene in untreated animals. Seven mice were fitted with silastic cannulae placed in the right atrium of the heart through the right jugular vein, as described by Harms and Ojeda [7]. Using a 12 in. length of silastic tubing (0.012 in. ID) connected to the cannula end protruding from the animals, we were able to administer both compounds to unanesthesized, unrestrained animals. Twenty-four hours after surgery, animals were connected to the leader tubing and were placed in a 1 liter Erlenmeyer flask. Following a 30 min habituation period, the mice received 10 μCi of [^{3}H]2DG in 10 μl, flushed into the heart with 50 μl of 0.9% saline. Forty min later, 5 μCi of [^{14}C]antipyrene in 5 μl was administered in the same manner. Ten seconds later, cardiac arrest was elicited with 100 μl of saturated KCl. Cannula placement was verified by dissection and the brain was perfused with 0.9% saline.

Uptake was examined in the following brain regions: olfactory bulbs, frontal cortex (areas 6 and 8), parietal cortex (areas 1, 3, and 4), occipital cortex (areas 17 and 18), olfactory tubercle, entorhinal cortex (areas 27, 49, and 35), cingulate cortex (areas 8, 24, 29), striatum, septal nuclei, hippocampus, amygdala, hypothalamus, thalamus, cerebellum, mesencephalon, and brain stem. Cortical areas were as designated for mouse brain by Caviness [2]. Brain pieces pooled from both hemispheres were weighed on a Sartorius analytical balance. A sample of whole trunk blood was also obtained at the time of sacrifice. All samples were placed in scintillation vials with 400 μl of Soluene 350 (Packard Instrument Co.). When digested, scintillation fluid was added and samples were counted in a Packard 2425 Liquid Scintillation Spectrometer. Correction for quench was performed by external standardization.

Biochemical analysis of the ^{3}H taken up was also performed. Of the ^{3}H present in the brain 45 min after subcutaneous injection, 9% was recovered in water and less than 0.5% in protein. Analysis of the remaining (trichloroacetic acid-soluble) radioactivity by paper chromatography (isopropanol:water, 4:1 (v/v)) revealed that 78% of the ^{3}H migrated in the position of 2DG-6-phosphate and 18% with 2DG. Following treatment of the TCA-soluble fraction with *E. coli* alkaline phosphatase (Worthington Bio-Chemical Corp.) at pH 8.0 for 60 min at 25°C, 89% of the ^{3}H comigrated with 2DG. We conclude that the bulk of brain ^{3}H was present in 2DG-6-phosphate with significant amounts in 2DG and water.

Plasma glucose concentrations were measured using an enzymatic assay (Calbiochem).

Tissue values are presented as dpm/mg tissue wet weight. These values were normalized by dividing the whole brain specific activities derived by summating the values for the dpm and the wet tissue weight of all pieces of each brain. This normalized uptake provided a measure of regional uptake relative to that of whole brain, allowing a comparison of regional differences independent of any main hormone effects.

Analyses of variance (ANOVA) were performed using a Statistical Analysis System (SAS) General Linear Mode (GLM) program on an Amdahl 470, version 6 computer on both the raw and ratio data for both experiments independently and for values derived from treatments that were common to both experiments. Variables considered were treatment, brain region, day of sacrifice and treatment-region interaction, and the particular experiment for those analyses that tested pooled treatments. We also performed analysis of variance upon the ratio value for each region independently.

RESULTS

The results of two separate footshock experiments were combined and are presented in Fig. 1. Whole brain 2DG

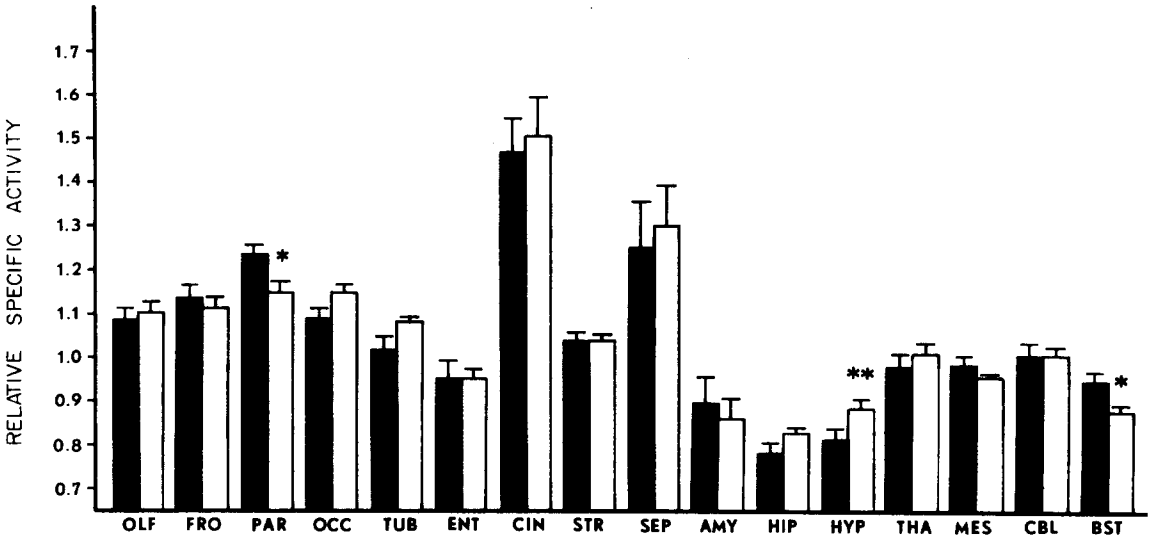

FIG. 1. The regional distribution of [^{3}H]2DG uptake following footshock compared with quiet controls. Bars represent the dpm/mg tissue divided by dmp/mg whole brain (mean ± SEM): Solid bars, Quiet animals; open bars, footshocked animals. OLF, olfactory bulb; FRO, frontal cortex; PAR, parietal cortex; OCC, occipital cortex; TUB, olfactory tubercle; ENT, entorhinal cortex; CIN, cingulate cortex; STR, striatum; SEP, septum; AMY, amygdala; HIP, hippocampus; HYP, hypothalamus; THA, thalamus; MES, mesencephalon; CBL, cerebellum; BST, brain stem. *Significantly different from Quiet, $p<0.02$. **$p<0.025$.

TABLE 1

WHOLE BRAIN [^{3}H]2DG UPTAKE

	ACTH								
	4-9[a]	4-10	4-10D[b]	1-24	α-MSH	LVP	COST[c]	SAL	Quiet
N	9	19	10	10	9	10	10	18	17
Brain 2 DG uptake (dpm/mg)	738 ± 100	614 ± 32‡	661 ± 38‡	592 ± 32‡	573 ± 25†	417 ± 22*	619 ± 53†	658 ± 34†	767 ± 50
Tissue/Blood[d]	5.6 ± 0.6	5.4 ± 0.4	5.1 ± 0.4	4.6 ± 0.2†	4.7 ± 0.2	3.6 ± 0.2*	5.7 ± 0.7	5.3 ± 0.2	5.6 ± 0.3

Values given are the mean dpm/mg tissue ± SEM

[a][Met_4SO_2,D-Lys_8,Phe_9]$ACTH_{4-9}$, [b][D-Phe_7]$ACTH_{4-10}$, [c]corticosterone, [d]Tissue dpm/mg divided by blood dpm/μl.

* Significantly different from Saline, $p<0.01$, † Significantly different from Quiet, $p<0.05$, ‡ Significantly different from Quiet, $p<0.01$

uptake was decreased 17% by footshock, but this effect was not statistically significant. Since the variance differed for different brain regions, analysis of variance was also performed independently for each brain region. The normalized [^{3}H]2DG uptake in parietal cortex and brain stem were both decreased 7% ($p<0.02$) by footshock, while that in the hypothalamus was increased 8.5% ($p<0.025$). No significant changes were detected in any other brain region.

The results of the two hormone experiments were also combined where appropriate. Table 1 shows the effects on whole brain [^{3}H]2DG uptake. Analysis of variance revealed that the treatment variable was highly significant ($F=3.89$, $p<0.0005$). A Duncan's New Multiple Range test using the raw (dpm/mg) data indicated that Quiet animals had significantly higher uptake than did all other treatments except $ACTH_{4-9}$ and [D-Phe_7]$ACTH_{4-10}$, and that the LVP treatment resulted in uptake significantly lower than saline. When the data were normalized by dividing the brain dpm/mg by the radioactivity of the whole blood, the differences in uptake between the Quiet animals and the other treatment groups was decreased; only the $ACTH_{1-24}$ and LVP groups were then significantly lower than Quiet. However, the LVP group remained significantly lower than saline.

This suggests that the altered 2DG uptake was due to a peripheral effect rather than a direct cerebral one. To test this, plasma ^{3}H and glucose were determined following $ACTH_{1-24}$, α-MSH, LVP, Saline and Quiet treatments. [^{3}H]2DG was injected 10 min after the hormone injection, and animals decapitated 10 min later. The specific activity of plasma glucose (i.e., [^{3}H]deoxyglucose per mg glucose) was significantly increased in the Saline as compared to the Quiet group (Table 2). Also, the LVP-treated group had a specific activity significantly below that of Saline.

Analysis of variance of the brain dpm/mg over all brain regions indicated a highly significant hormone effect ($F=61.53$, $p<0.001$). However, the interaction between hormone and region was not significant ($F=0.39$, $p\simeq1.000$), indicating that no region of the brain was selectively influenced by any hormone treatment. Analysis of variance for each region, independent of the other regions, revealed only

the treatment effects seen in the whole brain analysis.

A normalization of the data by whole brain radioactivity was performed to remove the overall effects of the treatments which may have been caused by peripheral factors. The results are presented in Table 3. Analysis of variance over all brain regions indicated that there was no longer a significant effect of treatment ($F=1.00$, $p\simeq0.40$). The hormone-region interaction was also not significant ($F=1.00$, $p\simeq0.40$). Analysis of variance for each region independently showed a significant difference only in the olfactory bulb. In this region, the Quiet group showed significantly lower uptake than the Saline, $ACTH_{4-10}$, and Corticosterone groups. Confidence intervals (95%), based upon the largest SEM of a given brain region ranged from 5–16% depending on brain region. Thus any changes that occurred following the hormone treatments were less than these confidence limits.

TABLE 2

EFFECT OF HORMONES ON PLASMA GLUCOSE AND 2DG SPECIFIC ACTIVITY

Treatment	Plasma Glucose mg/ml	Radioactivity dpm/mg glucose $\times 10^{-4}$
Quiet	1.04 ± 0.05	50.4 ± 3.6
Saline	1.20 ± 0.04	62.8 ± 7.2*
α-MSH	1.19 ± 0.07	55.0 ± 4.6
$ACTH_{1-24}$	1.19 ± 0.07	55.0 ± 4.4
LVP	1.30 ± 0.10	38.6 ± 2.4†

* Significantly different from Quiet, $p<0.05$

† Significantly different from $ACTH_{1-24}$, α-MSH and Saline, $p<0.05$

Goldman *et al.* [5,6] found that a α–MSH induced changes in antipyrene uptake, indicating changes of cerebral blood flow in rats. Since blood flow and cerebral metabolic rate are often considered to be related [21], we compared the regional uptake of [^{3}H]2DG and [^{14}C]antipyrene in the same (untreated) animals. Regional data were normalized by the summed whole brain radioactivity for each isotope in a given brain. Figure 2 shows the results of this experiment. It is readily apparent that there were substantial differences in the uptake of the two compounds. Most cortical regions tended to take up a relatively larger amount of deoxyglucose, whereas thalamus, cerebellum and brain stem took up a relatively larger amount of antipyrene. The highly significant differences in the ratios of uptakes among regions suggests that the two methods do not correlate as well as previously supposed [19].

DISCUSSION

Previous studies on 2DG uptake have used ablation or gross stimulation, especially of sensory systems and have shown dramatic changes in specific cerebral structures [16,21]. In contrast, the present studies were expected to show more subtle regional effects. Nevertheless, footshock altered the specific regional uptake of 2DG in the hypothalamus, parietal cortex and brain stem.

By contrast, the only significant regional change caused by the hormone injections was in the olfactory bulb. This unexpected result was not associated with concomitant changes in the olfactory tubercle. None of the hormones appeared to mimic the effects of footshock and thus the latter are unlikely to have been mediated by the hormones released during the stressful experience. The doses of hormones used

TABLE 3

HORMONE ADMINISTRATION AND RELATIVE REGIONAL [^{3}H]2-DEOXYGLUCOSE UPTAKE

	Rel. Dpm/mg		Percentage of Saline						
			ACTH						
Treatment Region	SAL N=18	QUIET 17	4-9 10	4-10 20	4-10D 10	1-24 21	MSH 9	LVP 10	COST 10
Olfactory Bulb	1.17 ± .03	92 ± 4*	94 ± 3	102 ± 3	100 ± 3	94 ± 3	95 ± 3	106 ± 5	110 ± 4
Frontal Cortex	1.48 ± .08	93 ± 1	93 ± 4	96 ± 3	93 ± 4	93 ± 3	100 ± 4	97 ± 4	87 ± 3
Parietal Cortex	1.47 ± .02	98 ± 2	97 ± 4	98 ± 2	95 ± 3	98 ± 3	100 ± 2	96 ± 3	100 ± 3
Occipital Cortex	1.27 ± .03	97 ± 3	101 ± 4	103 ± 2	97 ± 3	102 ± 2	95 ± 3	108 ± 5	99 ± 4
Olfactory Tubercle	1.25 ± .05	100 ± 2	96 ± 3	97 ± 3	100 ± 3	101 ± 3	97 ± 3	101 ± 3	92 ± 3
Entorhinal Cortex	.93 ± .03	104 ± 2	101 ± 4	104 ± 2	94 ± 4	107 ± 3	97 ± 3	110 ± 5	104 ± 3
Cingulate Cortex	1.44 ± .04	103 ± 2	95 ± 5	106 ± 4	94 ± 5	104 ± 2	97 ± 4	101 ± 3	100 ± 2
Striatum	1.01 ± .02	102 ± 2	104 ± 2	100 ± 2	100 ± 1	102 ± 2	102 ± 2	101 ± 2	99 ± 2
Septum	.88 ± .04	107 ± 5	96 ± 4	102 ± 3	97 ± 3	105 ± 3	99 ± 3	114 ± 4	114 ± 4
Hippocampus	.77 ± .02	97 ± 2	99 ± 2	98 ± 2	97 ± 2	99 ± 2	99 ± 2	96 ± 3	94 ± 2
Amygdala	.79 ± .04	113 ± 7	95 ± 7	104 ± 5	103 ± 6	109 ± 5	117 ± 9	111 ± 5	108 ± 11
Hypothalamus	.76 ± .02	101 ± 2	97 ± 2	101 ± 3	101 ± 3	105 ± 2	98 ± 3	108 ± 5	101 ± 3
Thalamus	.95 ± .02	104 ± 2	104 ± 2	98 ± 1	103 ± 2	99 ± 2	96 ± 2	99 ± 3	99 ± 3
Mesencephalon	.96 ± .02	96 ± 4	100 ± 2	94 ± 1	99 ± 2	96 ± 1	100 ± 2	92 ± 5	93 ± 3
Cerebellum	.99 ± .03	100 ± 2	104 ± 3	100 ± 2	108 ± 2	100 ± 2	101 ± 4	96 ± 2	96 ± 3
Brain Stem	.90 ± .02	105 ± 2	101 ± 1	100 ± 1	102 ± 3	100 ± 2	103 ± 1	106 ± 2	103 ± 2
Experiment	1 and 2	1 and 2	2	1 and 2	2	1 and 2	2	1	1

The values for Saline treatment are the mean ± SEM of the dpm/mg tissue divided by the dpm/mg whole brain. For the remaining treatments this same value is expressed as the percentage (±SEM) of the Saline group for the same region from the same experiment(s).

* Significantly different from Saline, $p<0.01$ (ANOVA).

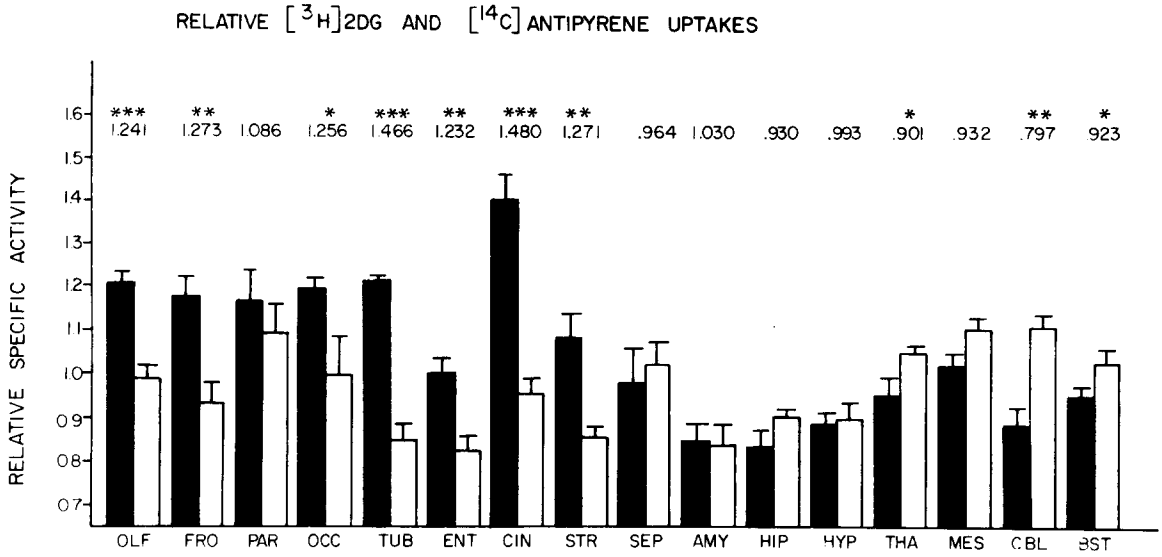

FIG. 2. A comparison of the uptakes of [^{3}H]2-deoxyglucose and [^{14}C]antipyrene. Solid bars represent the ^{3}H dpm/mg tissue for each region divided by that for whole brain. Open bars represent the same ratio for ^{14}C. The numbers are the ratio of these two values for each brain part. Brain parts are designated as in Fig. 1. *The normalized antipyrene uptake was significantly different from the 2DG uptake ($p<0.05$). **$p<0.01$. ***$p<0.001$.

are those that have been reported previously to have significant behavioral effects [27]. Thus if these hormones do have any effect upon the brain metabolism, they must affect the whole brain, or must alter brain metabolic rate at a more local level than our dissections could discern, or the effect is smaller than our 5–16% confidence interval.

Goldman *et al.* [5,6] reported that α–MSH (0.04 μg/g, IV) decreased blood flow in many brain regions of the rat 5, 10 and 20 min after injection, but spared occipital cortex at all times. In the present study, α–MSH was administered subcutaneusly to mice at a dose of 0.5 μg/g, and [^{3}H]2DG 10 min later. Since the major part of 2DG uptake occurs in the first 10 min following injection [16], and since the higher dose and route of injection would be expected to prolong the action of the hormone, we expect that 2DG uptake occurred in our animals during the period of altered blood flow reported by Goldman. However, we did not observe any regionally specific effect of α–MSH on 2DG uptake.

Blood flow and glucose uptake have been reported to be parallel indices of cerebral metabolism [19], although it is entirely possible that changes in blood flow could occur without concomitant changes in glucose uptake. To test the parallelism between blood flow and glucose uptake we studied the uptake of [^{3}H]2DG and [^{14}C]antipyrene in the same animals. Our results (Fig. 2) indicated highly significant differences among brain regions, with cortical areas generally showing increased uptake of 2DG relative to antipyrene, while the reverse was true in subcortical regions. These results are in direct conflict with those of Reivich and Sokoloff [19]. However, it is interesting that in rat striatum, Sokoloff [21] found that amphetamine slightly but not significantly decreased 2DG uptake, whereas Lavyne *et al.* [13] found that amphetamine (1.5 mg/kg) caused a 33% increase in blood flow, as measured by the hydrogen clearance technique.

It is important to note that because different pulse times are required for [^{14}C]antipyrene and [^{3}H]2DG, the mice are not in exactly the same physiological state in the two procedures. We observed that the animals, which normally appeared somonolent, were more active for a short time following the injections. Thus, the antipyrene uptake was measured exclusively during a period of activity, whereas mice were active for only a very small fraction of the 2DG uptake. This problem is inherent in any comparison of the two methods and is not confined to studies done in the same animals; the antipyrene uptake is, of necessity, more temporally associated with injection than the 2DG uptake. However, there is no basis for suspecting that subcortical structures are less active than cortical ones during periods of inactivity. Our data from footshocked animals did not show such an effect.

The lack of effect of ACTH and its analogs on the striatum is surprising in view of our earlier findings of an effect of ACTH on dopamine turnover [10]. However, Sokoloff [21] recently reported only a small (nonsignificant) decrease of 2DG uptake in this structure following amphetamine administration. It is also noteworthy, that the effects on dopamine synthesis were observed after three daily injections of ACTH, whereas effects of acute injections have not yet been performed by us or reported by others. However, the effects on 2DG uptake observed in the footshock experiments may have been related to norepinephrine, the turnover of which is known to be activated by such stressors [24].

The treatment related changes we found in whole brain 2DG uptake are probably due to changes in the periphery. Our findings that these hormones alter the specific activity of plasma glucose support this idea. Consistent with our data, it has previously been shown that injection stress [1] and LVP [4] increase plasma glucose concentrations.

We suggest that either the hormones at the doses we

tested do not affect the regional metabolic rate, or that the consequent changes in brain metabolic rate are smaller or more discrete than our methods allowed us to detect. Autoradiographic procedures which have much finer anatomic resolution might reveal important but very localized changes.

ACKNOWLEDGEMENTS

We are grateful to Drs. H. Greven and H. Van Riezen of Organon International for the provision of α-MSH and the ACTH analogs. This research was supported by USPHS grant number MH 25486. Richard L. Delanoy is an NSF predoctoral fellow. Adrian J. Dunn is a Sloan Foundation Fellow in Neurobiology. The technical assistance of Nancy Gildersleeve, Carol Underwood and Leila Baz is greatly appreciated.

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