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Glucosamine Incorporation into Rat Cerebrum: Effect of Adrenalectomy, Corticosterone, Exercise, and Training[1]

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IRWIN, L. N. AND D. M. TERRIAN. *Glucosamine incorporation into rat cerebrum: Effect of adrenalectomy, corticosterone, exercise, and training.* PHARMAC. BIOCHEM. BEHAV. 9(1) 33–37, 1978.—Incorporation of D-[l-^{14}C]glucosamine into various metabolic fractions was studied in an experiment designed to quantify the relative influence of physiological and behavioral factors. Different physiological states were established by sham operation (S), adrenalectomy (A), and adrenalectomy plus corticosterone replacement (H). Within each physiological condition the behavioral state was varied by swim-escape training (E), swimming exercise (X) or nonswimming controls (C). Adrenalectomy caused a generalized increase in label uptake by cerebral cortex and hippocampus, but precursor levels in the blood were elevated also, suggesting a systemic physiological effect. Behavioral state had no effect on overall uptake, but did influence the distribution of label between soluble and membrane-bound glycoproteins. These results indicate that D-[l-^{14}C]glucosamine is an effective glycoprotein and ganglioside precursor in behavioral experiments, provided corrections for the influence of systemic physiological factors are made.

[^{14}C]Glucosamine Glycoproteins Carbohydrate metabolism Corticosterone Exercise
Swim-escape training Stress Cerebral cortex Hippocampus

GLUCOSAMINE has commonly been used in metabolic studies of glycoproteins and glycolipids because of its ready incorporation into these macromolecules as hexosamines and sialic acid [1, 7, 8]. Although behavioral stimulation has been reported to alter incorporation of glucosamine into macromolecules in some cases [4, 9, 11], the elements of behavioral experience directly responsible for changes in glucosamine uptake are difficult to pinpoint because of the systemic physiological adjustments that accompany the behavioral manipulation [13]. For example, recent findings [5,10] that hormones of the pituitary-adrenal axis influence protein synthesis and catecholamine turnover in brain raise the question of whether metabolic changes during behavioral experience relate directly to the processing of task-specific information or to hormonal fluctuations induced by exercise, arousal, or other generalized physiological processes. This question cannot be answered until the relative influence of physiological and behavioral factors is systematically investigated. Therefore, we designed an experiment in which physiological and behavioral conditions were varied so that the contribution of each to any changes in glucosamine incorporation could be determined quantitatively.

METHOD

Experimental Design

Male albino rats, 30–33 days of age weighing an average of 155 g, were obtained from Holtzman (Madison, Wisconsin) and housed under constant photoperiod and temperature with food and water available ad lib. Eight rats were randomly assigned to each of nine subgroups in a 3 × 3 factorial design consisting of three physiological conditions and three behavioral situations (Fig. 1).

Physiological manipulation. Two-thirds of the rats were adrenalectomized bilaterally and one-half of these received a subcutaneous implant of a silicone rubber capsule (Dow Corning, Silastic, 2.4 mm diameter, 30 mm long) packed with crystalline corticosterone to provide a slow constant release of hormone into the bloodstream [3]. The remaining one-third were sham operated. The sham-operated (Group S), adrenalectomized (A) and hormone replacement (H) rats were maintained in the animal room for seven days and moved in their home cages to a new room for radioisotope injection and behavioral treatment the eighth day. A solution of 0.09% NaCl was provided as drinking water for Group A

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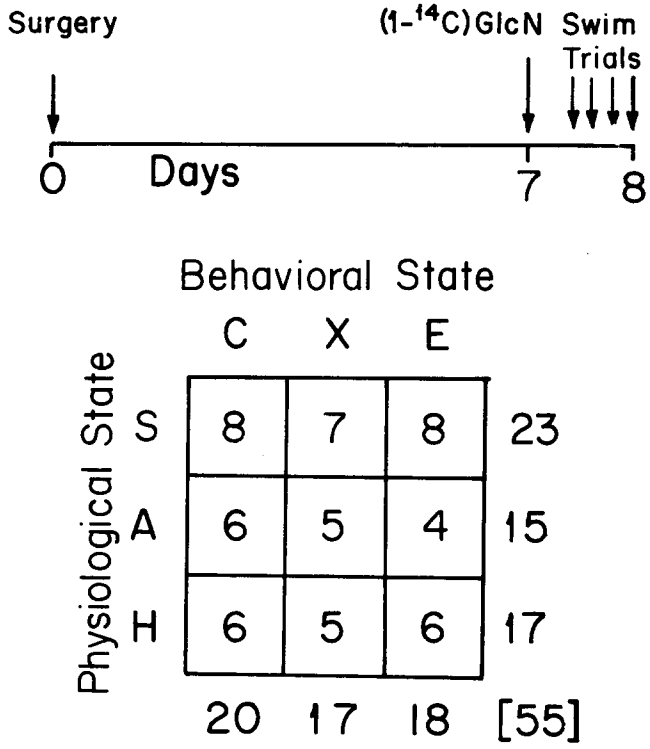

FIG. 1. Experimental design and number of survivors. Rats were sham operated (S), adrenalectomized without hormone replacement (A), or adrenalectomized with corticosterone replacement (H) on Day 0. Seven days later, 10 μCi D-[l-^{14}C]glucosamine HCl was injected subcutaneously. Each physiological group was subdivided into cage control (C), exercise (X), or swim-escape (E) behavioral groups. Rats were handled (Group C) or given swimming trials (Groups X and E) at 8, 6, 4, and 2 hr prior to sacrifice 24 hr postinjection.

following surgery. The primary purpose of these manipulations was to alter systemic carbohydrate metabolism, which was monitored by assays for blood glucose and liver glycogen [14]. All silicone rubber implants retained some corticosterone at autopsy, indicating hormone release throughout the experimental period.

Behavioral manipulation. Each of the physiological groups are subdivided into three behavioral categories. Rats in one category (Group E) learned to escape from cold (22°C) water 20 cm deep in a No. 3 galvanized tub by climbing a rope suspended at the center of the pool. Rats were dropped into the water facing away from the rope and allowed to swim for 3 min or until they escaped. Four swimming trials were given at 2 hr intervals on the eighth postoperative day, beginning 8 hr prior to sacrifice. For each Group E rat, another rat was exposed to the same experimental situation, except that the rope was removed and the rat was lifted out of the water by hand after swimming as long as the Group E rat with which it was matched. Rats in this category (Group X) thus received the same amount of stress and exercise as rats in Group E, but did not learn how to escape by their own efforts. Rats in a third category (Group C) were handled briefly at 2 hr intervals corresponding to the swimming trials of their littermates, but otherwise were left undisturbed in their cages. The behavioral components and important parameters of this swim-escape task have recently been analyzed in detail [2].

Radioisotope Injection

D-[l-^{14}C]glucosamine-HCl (3 mCi/m mol) from Amersham-Searle was diluted in distilled water to a concentration of 100 μCi/ml. Each rat was injected subcutaneously with 0.1 ml (10 μCi) 24 hr prior to sacrifice. Incorporation of glucosamine into brain macromolecules reaches asymptotic levels between 12 and 24 hr postinjection [4,9].

Tissue Samples and Biochemical Fractionation

Rats were killed by decapitation, and samples of blood, the parietal region of the cerebral cortex, and the whole hippocampus were collected. Aliquots of blood were transferred immediately to assay tubes for glucose determinations and to counting vials for measurement of radioactivity. The brain tissues were frozen in crushed dry ice and lyophilized.

Glucosamine is taken up by the cell into both particulate fractions (mostly membrane-bound glycoproteins and gangliosides, and some nonpolar lipids) and soluble fractions (soluble glycoproteins and unincorporated glucosamine) [6–8]. Therefore, brain samples were fractionated into these five general metabolic pools, as follows: Lyophilized tissue weighing about 50 mg was homogenized in 3 ml 10 mM NH_4HCO_3 with a Potter-Elvehjem apparatus, diluted to 10 ml, and centrifuged at 100,000 g for 1 hr. The pellet was resuspended and centrifuged as before. The combined supernates were lyophilized then resuspended in 3 ml 5% trichloroacetic acid—0.25% phosphotungstic acid at 4°C for 30 min, then centrifuged at 12,000 g for 10 min. The pellet was resuspended in acid and spun down again. The combined supernates constituted the acid-soluble fraction of small molecules and unincorporated label. The pellet consisted of precipitated soluble macromolecules.

The 100,000 g pellet from the homogenate was transferred to a sintered glass filter, moistened with 0.1 M KCl, and stirred in 2 ml hot (~60°C) chloroform:methanol (1:1, v/v) for 5 min. The liquid was drawn through the filter by vacuum and collected in a flask. The residue was extracted 4 more times with 2 ml and a fifth time with 5 ml of chloroform:methanol as before, leaving a lipid insoluble residue consisting primarily of membrane proteins and glycoproteins. The total lipids in the combined chloroform:methanol extracts were separated into gangliosides and nonpolar lipids by a silicic acid adsorption method [12].

Measurement of Radioactivity

A commercial scintillation cocktail (ACS, Amersham-Searle) was added directly to aliquots of the liquid fractions (deproteinated blood, acid-soluble supernate, nonpolar lipids, and gangliosides). The lipid insoluble residue was moistened with 0.2 ml H_2O and dissolved overnight in a commercial solubilizer (NCS, Amersham-Searle), while the precipitated soluble macromolecules were dissolved in 1 N NaOH, prior to addition of ACS, then adjusted with 0.1 ml glacial acetic acid. Samples were counted by liquid scintillation with an efficiency of 82–96% as determined by internal standards. All counts are corrected to 100% efficiency and reported as disintegrations per minute (dpm).

Statistical Analysis

Two-way analysis of variance was run on all the data collected in this study, since a primary concern was detection of statistical interaction between the physiological and

behavioral variables. However, in no case was a significant interaction between the two main effects found, so the data were pooled according to physiological state and behavioral state independently and are thus presented in this report. All statistical probabilities are based on two-tailed t tests.

RESULTS

Adrenalectomy resulted in significantly lowered blood glucose levels and a severe depletion of liver glycogen. Corticosterone replacement prevented the drop in blood glucose but did not block liver glycogen depletion (Table 1). All adrenalectomized rats showed typical torpor and muscle weakness. Despite saline in the drinking water, mortality among adrenalectomized rats was rather high (36%), primarily due to respiratory infections.

Despite gross differences in spontaneous activity and general physiological condition, all escape-trained (Group E) rats learned the swim escape task in typical 1-trial fashion. Latencies for the first (learning) trial were greater in the adrenalectomized and corticosterone-replacement rats, but by the fourth trial the mean latencies for all three physiological treatments were less than 15 sec. Thus, the experimentally-induced changes in systemic physiology did not significantly impair performance of the behavioral task.

Analysis of variance showed physiological state to have a significant effect on total uptake of labelled glucosamine in all tissues examined. The detailed breakdown (Table 2) shows uptake by cerebral cortex and hippocampus to be significantly higher in Group A, and somewhat higher in Group H than in Group S. The amount of labelled glucosamine in the blood at the time of sacrifice is significantly higher in both Groups A and H than in Group S.

Behavioral state had no significant effect on overall glucosamine uptake (Table 2). In most cases, uptake tended to be lower in escape-trained (Group E) and exercised (Group X) rats than in control (Group C) rats, as reported previously [9], but the differences were not statistically significant in the present experiment because of the large variance attributable to the different physiological states.

Because physiological state clearly influenced the amount of glucosamine entering the brain, data on the distribution of label within different metabolic pools had to be normalized to an assumed constant amount of precursor. This was accomplished by expressing the amount of radioactivity in any single metabolic fraction as a percentage of the total radioactivity in all metabolic fractions from that tissue sample. When normalized in this way, the data reveal no effect of physiological state on relative distribution among the five metabolic fractions studied (Fig. 2). However, behavioral state caused a slight shift in at least one fraction, as escape-trained (Group E) rats incorporated significantly less label than control (Group C) rats into the lipid-insoluble residue, with exercised (Group X) rats incorporating an intermediate amount (Fig. 3). A reciprocal pattern of incorporation (highest in Group E, lowest in Group C) among the three groups is seen in the soluble macromolecules, though the small differences are not statistically significant.

TABLE 1

EFFECT OF SURGERY AND HORMONE REPLACEMENT ON CONTENT OF BLOOD GLUCOSE AND LIVER GLYCOGEN

Group	Blood Glucose (mg/100ml blood)	(%Δ	Liver Glycogen (μg glucose/mg)	(%Δ)
S	97.5 ± 2.1		311 ± 32	
A	84.7 ± 4.2	−13*	31 ± 8	−90†
H	93.3 ± 2.9	−4	49 ± 14	−84†

Values are given as $\bar{x}$ ± SEM Percent change (%Δ) is calculated relative to the value for Group S.
* different from Group S at $p<0.01$
† different from Group S at $p<0.001$

TABLE 2

EFFECT OF PHYSIOLOGICAL AND BEHAVIORAL MANIPULATION ON [1-^{14}C]GLUCOSAMINE UPTAKE INTO RAT CEREBRAL CORTEX, HIPPOCAMPUS, AND BLOOD

Tissue	Physiological Manipulation Group	Radioactivity	%Δ	Behavioral Manipulation Group	Radioactivity	%Δ
Cerebral	S	218 ± 14 (17)		C	253 ± 16 (15)	
Cortex	A	259 ± 11 (15)	+19*	X	222 ± 9 (17)	−12
	H	241 ± 11 (16)	+11	E	242 ± 12 (16)	−4
Hippo-	S	129 ± 6 (20)		C	141 ± 8 (17)	
campus	A	157 ± 10 (13)	+22†	X	147 ± 9 (12)	+4
	H	141 ± 8 (15)	+9	E	136 ± 7 (19)	−4
Blood	S	572 ± 71 (23)		C	757 ± 92 (18)	
	A	733 ± 76 (15)	+28‡	X	590 ± 68 (17)	−22
	H	777 ± 78 (17)	+36‡	E	685 ± 68 (20)	−10

Radioactivity is calculated as dpm/mg dry weight brain or dpm/0.1 ml blood, and expressed as mean ± SEM (n). Percent change (%Δ) is computed for adrenalectomized (A) or corticosterone replacement (H) relative to sham operated (S) rats, and for exercised (X) or swim escape (E) relative to cage control (C) rats.
* differs from Group S at $p<0.05$
† differs from Group S at $p<0.01$
‡ differs from Group S at $p<0.001$

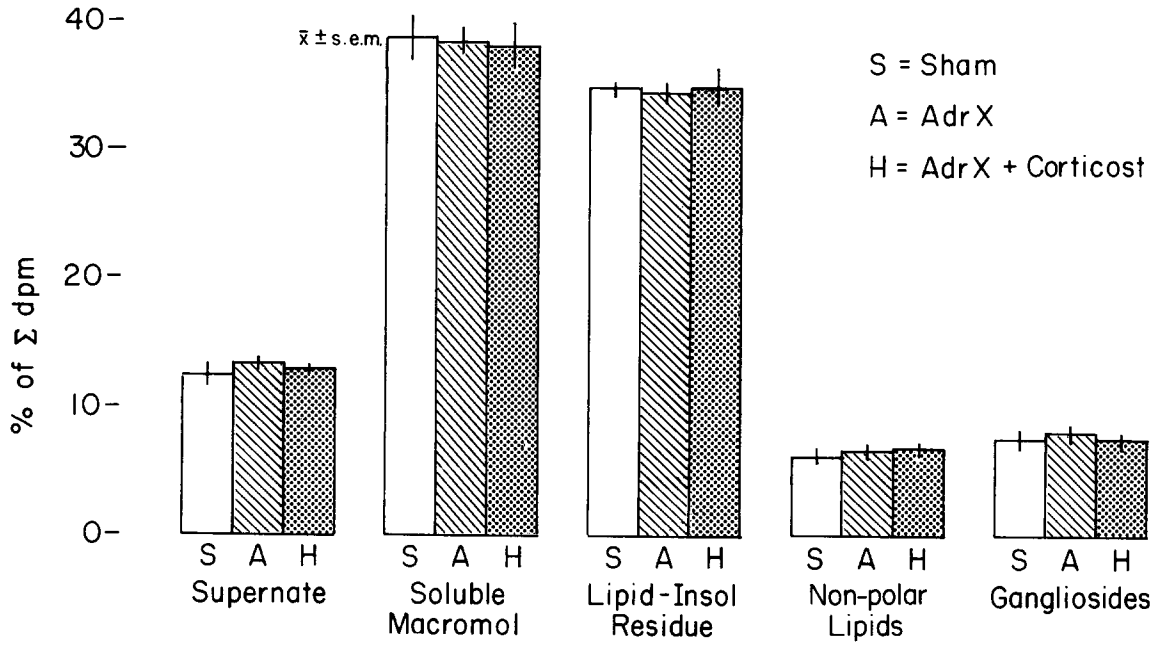

FIG. 2. Effect of sham operation (S), adrenalectomy (A), and adrenalectomy with corticosterone replacement (H) on distribution of radioactivity among acid-soluble supernate (primarily small molecules), acid-precipitable soluble macromolecules (primarily as soluble glycoproteins), lipid-insoluble residue (primarily membrane-bound glycoproteins), nonpolar lipids including some phospholipids and cerebrosides, and gangliosides from rat cerebral cortex. Tissue was collected 24 hr after subcutaneous injection of 10 μCi D-[l-^{14}C]glucosamine. Radioactivity is expressed as percent counts in each fraction of total counts in all five fractions.

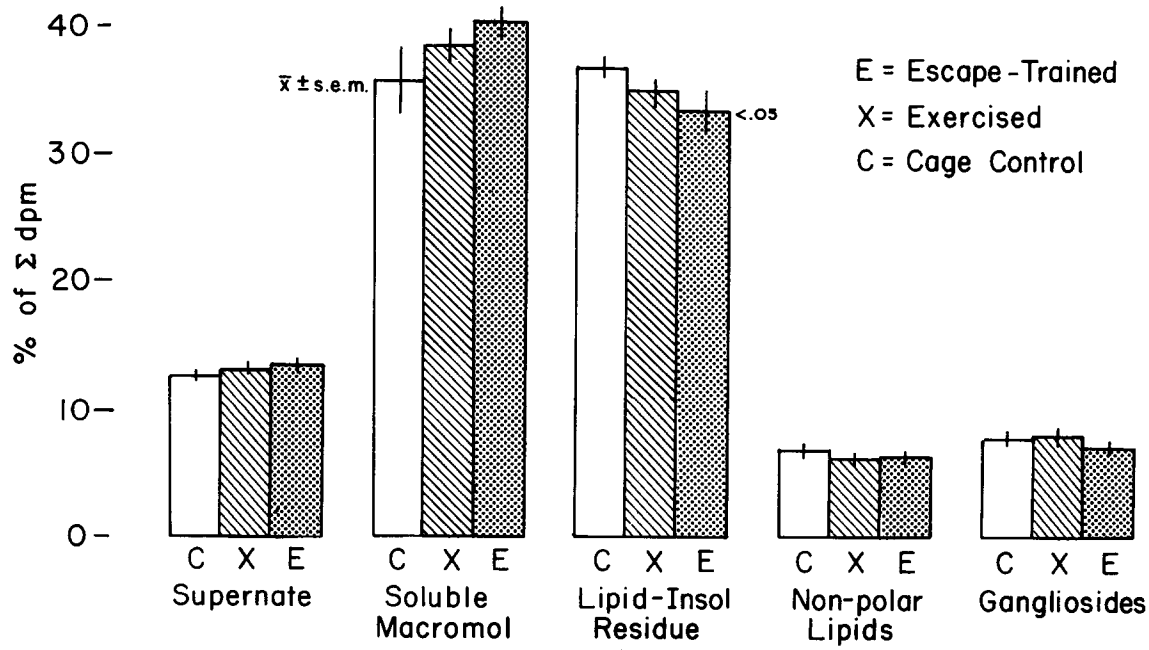

FIG. 3. Effect of swim-escape (E), exercise (X), or cage control (C) conditions on distribution of radioactivity from D-[l-^{14}C]glucosamine, determined as in Fig. 2. Value for Group E is significantly lower ($p<0.05$) than for Group C in the lipid-insoluble residue.

DISCUSSION

Most attempts to correlate biochemical events with behavior are confounded by physiological perturbations associated with the different behavioral states in the experiment [13]. These physiological fluctuations may be responsible for observed biochemical changes in the brain, irrespective of the information processing associated with the behavioral adjustment itself. The aim of this experiment was to vary the state of the animal simultaneously along two dimensions—one physiological, the other behavioral—and determine the relative contribution of each factor to variation in a metabolic endpoint (glucosamine incorporation) of significance in behavioral neurochemistry research. Our major finding is that physiological and behavioral manipulation both affect glucosamine uptake by brain cells but in different and distinguishable ways.

Physiological manipulation affected the overall level of glucosamine uptake by brain cells, as shown by the greater incorporation of label into both neocortical and hippocampal samples of adrenalectomized rats than from sham-operated or corticosterone-replacement rats. The adrenocortical system was chosen for manipulation because of its crucial role in the homeostatic control of carbohydrate metabolism, and the fact that blood levels of radioactivity were also elevated in adrenalectomized animals suggests that the differences in uptake by brain cells were a consequence of differences in peripheral utilization, hence cerebral availability, of glucosamine.

Despite the apparent severity of changes in the systemic physiology of adrenalectomized animals, as shown by muscle weakness and significantly lowered blood glucose and liver glycogen levels, the distribution of glucosamine among major metabolic pools was not different from that of the sham-operated or corticosterone-replacement rats, after adjustment for the differences in overall uptake (Fig. 2). Hence, glucosamine, once it enters the cell, must be shunted to its various metabolic destinations irrespective of the energy mobilization requirements of the body as a whole. These results imply that glucosamine is a good precursor to use in behavioral studies that might differentially affect energy turnover, provided that compensation is made for differences in overall tissue uptake.

Behavioral manipulation did not affect overall uptake significantly (Table 2) but appeared to result in a slight redistribution of glucosamine among the major intracellular metabolic pools (Fig. 3). The decrease of label from the lipid-insoluble pool of brain cells from escape-trained rats, though small, was statistically significant, and replicates a previous result [9]. A smaller but nonsignificant trend was seen in brains of exercised rats as well. A depletion of label in this pool reflects lower net incorporation into membrane-bound glycoproteins, since soluble glycoproteins, glycolipids, and precursor molecules are extracted into other fractions.

No statistical interaction between the effects of physiological and behavioral manipulation was observed in this experiment, but the possibility of such interaction in other experimental situations can not be precluded. Even if no statistical interactions occur, the net metabolic effect, as shown by this experiment, must be considered a composite of systemic physiological (e.g., hormonal) factors and intrinsic neural/behavioral influences. Of course, the distinction between extrinsic and intrinsic influences becomes arbitrary at the cellular level, but an experimental design such as the one used here allows the quantification of the relative contribution of operationally defined extrinsic (physiological) and intrinsic (behavioral) components.

In summary, we have demonstrated that systemic physiology influences the access of glucosamine to the initial intracellular precursor pool, but if normalized for these nonspecific physiological influences, the distribution of glucosamine among various metabolic fractions remains stable and predictable. On the other hand, behavioral manipulation may influence the intracellular metabolic fate of glucosamine once it has entered the precursor pool.

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