Effects of Corticoids, Insulin, and Epinephrine on α-Aminoisobutyrate Accumulation in Muscle of Adrenalectomized Rats.* (26473)

J. EICHHORN, M. FEINSTEIN, I. D. K. HALKERSTON AND O. HECHTER
Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Insulin has been shown to increase the accumulation of the "non-utilizable" amino acid, α-aminoisobutyrate (AIB) *in vitro* in diaphragm muscle from intact rats(1) and *in vivo* using functionally nephrectomized, hypophysectomized rats(2). Early in the present work it was noted that the distribution of AIB in muscle of adrenalectomized rats after administration of insulin is about half of that observed in muscles of intact or hypophysectomized rats after insulin. Accordingly, the effects of epinephrine, deoxycorticosterone (DOC), and cortisol administration upon muscle AIB accumulation in adrenalectomized rats with and without the administration of insulin have been studied. The results show that corticoids and epinephrine, independently of each other, enhance the effect of insulin upon accumulation of AIB in muscle of adrenalectomized rats.

Methods. Intact, hypophysectomized (2 day), or adrenalectomized (6 day) rats of Charles River SD strain, weighing 120-190 g, were used. Adrenalectomized rats were maintained with 0.9% saline as drinking water. The effect of insulin administration on AIB transport was determined in diaphragm and gastrocnemius muscle in each of these 3 types of animals. Transport of AIB was also studied in these muscles of adrenalectomized rats in absence and presence of insulin after acute administration of epinephrine, and after pretreatment with DOC or cortisol.

The experimental procedures, described previously(2,3), involved functional nephrectomy under Avertin anesthesia, followed by subcutaneous injection of AIB-1-C^{14}, 1.5-2.5 μc in 0.3 ml water (Volk, S.A. 2.26 μc/mg). DOC and cortisol were suspended in Special Formula No. 17874 (Upjohn), and administered subcutaneously to adrenalectomized rats under 2 schedules: (a) "maintenance-type" dosage, 0.8 mg each day for 6 days, beginning on day of adrenalectomy with none on day of experiment (total, 4.8 mg/rat); (b) short-term dosage in three injections (1.6 mg ea) 6, 4, and 2 hr before sacrifice on the 7th day after adrenalectomy (total, 4.8 mg/rat). 1-epinephrine bitartrate (100 μg/rat, equivalent to 55 μg epinephrine) was injected subcutaneously to adrenalectomized rats immediately after functional nephrectomy at a site distant from the subsequent AIB injection. Each group of rats (intact, hypophysectomized, adrenalectomized, and adrenalectomized treated with either epinephrine or corticoids) was divided so that part received insulin (1U/100 g) intraperitoneally accompanied by 100 mg glucose/100 g to prevent hypoglycemia; the remainder of each group received 25 mg glucose/100 g as a control injection.

Rats were reanesthetized (Nembutal) 90-100 min after AIB injection (which was also 90-100 min after epinephrine or insulin administration); plasma was obtained immediately from aortic blood, and tissues (diaphragm and gastrocnemius muscle) were excised without delay. Tissues were extracted with water at or near 100° for 15 min. The diluted (1:50) plasma and tissue extracts were plated and counted in a gas flow chamber. A previous report demonstrates that the radioactivity measured in this manner represents primarily unchanged AIB(2). Dosage of AIB used gave a concentration in plasma of 0.1 to 10 μg/ml, which is within the range previously found to yield distribution values in diaphragm which are relatively independent of alterations in plasma concentration (2).

Results. In Table I, the percentage distribution of AIB between tissues and plasma is expressed as the ratio, 100 × cpm per g wet tissue/cpm per ml plasma. AIB distributions in both muscles of untreated intact rats are significantly higher ($p = < .001$) than those in hypophysectomized or adrenal-

* This work is supported by U.S.P.H.S., Nat. Science Foundation, and the Commonwealth Fund.

TABLE I. Percent Distribution of AIB between Muscles and Plasma of Intact, Hypophysectomized, or Adrenalectomized Rats, and Influence of Various Hormones.

	Hormone treatment*		AIB: $\frac{\text{cpm/g wet tissue}}{\text{cpm/ml plasma}} \times 100$	
	Insulin	Other	Diaphragm	Gastrocnemius
Intact	—		157 ± 12 (18)†	93 ± 6 (4)†
	+		385 ± 44 (6)	121 ± 13 (7)
Hypophysectomized (2 day)	—		72 ± 7 (5)	41 ± 3 (5)
	+		420 ± 110 (16)	102 ± 13 (5)
Adrenalectomized (6 day)	—		91 ± 13 (12)	38 ± 5 (12)
	+		194 ± 6 (19)	51 ± 3 (19)
	—	Epinephrine	87 ± 9 (8)	55 ± 4 (8)
	+	"	354 ± 36 (9)	99 ± 12 (10)
	—	Cortisol (1-6 day)	154 ± 23 (10)	59 ± 8 (9)
	+	"	280 ± 30 (13)	75 ± 8 (13)
	—	DOC (1-6 day)	144 ± 12 (3)	72 ± 5 (3)
	+	"	497 ± 62 (5)	144 ± 16 (5)
	—	Cortisol (7th day)	67 ± 4 (10)	34 ± 3 (10)
	+	"	147 ± 16 (11)	50 ± 5 (12)
	—	DOC (7th day)	89 ± 10 (6)	39 ± 4 (6)
	+	"	192 ± 28 (6)	43 ± 5 (6)

* Dosage of hormone: Insulin, 1 U/100 g body wt; glucose with insulin, 100 mg/100 g; control glucose, 25 mg/100 g; epinephrine, 55 γ/rat; DOC and cortisol, total of 4.8 mg/rat in divided doses as described in text.

† Mean, stand. error, and No. of observations.

ectomized rats; no significant difference exists between the latter two groups. Administration of insulin increases AIB distribution about 2.5 times in diaphragm of intact rats, and 5.8 times in diaphragm of hypophysectomized rats; the respective increases in gastrocnemius are 1.3 times and 2.5 times the control level. In adrenalectomized rats, AIB distribution after insulin administration is increased 2.1 times in diaphragm, and 1.3 times in gastrocnemius. While the percentage increase in AIB distribution produced by insulin administration in muscle of adrenalectomized rats is approximately equivalent to the response in intact rats, the absolute level of AIB distribution achieved in adrenalectomized rats is about half that in muscle of intact rats. In hypophysectomized rats, where the level of AIB distribution in absence of exogenous insulin is statistically equivalent to that in untreated adrenalectomized rats, insulin increases AIB distribution in both muscles to values comparable to those in intact rats. Exogenous epinephrine alone in the dosage used has no effect in diaphragm, but slightly increases AIB distribution in gastrocnemius ($p = <.02$) of the adrenalectomized rats. However, when epinephrine is given in addition to insulin, the absolute levels of AIB distribution in both muscles are increased significantly above those obtained with insulin alone ($p = <.001$), giving values which are not significantly different from those after insulin treatment in intact or hypophysectomized rats.

"Maintenance-type" dosage with either cortisol or DOC in absence of exogenous insulin results in AIB distribution values in diaphragm comparable to those in untreated intact rats; these corticoids significantly increase the AIB distribution values in gastrocnemius above the untreated adrenalectomized level, but not to that in the untreated intact rat. After insulin administration to adrenalectomized rats treated with either DOC or cortisol for 6 days, AIB distributions in diaphragm are increased to a level which is statistically indistinguishable from values obtained in diaphragm of intact rats treated with insulin. However, the absolute level of AIB distributions with DOC plus insulin is significantly greater in diaphragm than with identical doses of cortisol plus insulin ($p = <.01$). In gastrocnemius, treatment with

either corticoid increases the level of AIB distribution over the non-treated adrenalectomized control; as in diaphragm, insulin administration increases the absolute AIB distribution values to a significantly greater extent in DOC-treated rats than in cortisol-treated rats. When given on the "short-term" basis, beginning 6 hr before sacrifice, neither corticoid elevated the distribution of AIB in either muscle in absence or presence of exogenous insulin.

Discussion. The present study demonstrates that, while insulin administration produces an increase in AIB distribution in muscle of adrenalectomized rats which on a percentage basis is equivalent to the insulin-induced increase in muscle of intact rats, the absolute level of muscle AIB distribution after insulin is much reduced in adrenalectomized rats relative to intact or hypophysectomized rats. The contrast between the effect of insulin in adrenalectomized and hypophysectomized rats is especially striking, since the values obtained in muscles of untreated rats in these 2 groups are about equivalent. Thus, in hypophysectomized rats, the response to insulin in muscle is markedly increased over adrenalectomized rats both on the basis of percentage change after insulin and in terms of absolute level of AIB distribution achieved. It thus appears that presence of the adrenal is required for the maximal effect of a given dosage of insulin on AIB accumulation in muscle.† Either epinephrine or maintenance-dosage with corticoids, independently of each other, clearly enhances the AIB accumulation effect of insulin in muscle, and it appears that both the adrenal cortex and medulla are implicated in the lower AIB distribution observed after insulin administration to adrenalectomized rats.

Since exogenous epinephrine augments the action of injected insulin to increase muscle AIB accumulation in adrenalectomized rats, it is possible that the elevated response to injected insulin observed in muscle of the intact and hypophysectomized rats relative to adrenalectomized rats is dependent in part upon the release of endogenous epinephrine induced by operative stress and/or insulin administration. Noall *et al.*(6) report a tendency for epinephrine to increase AIB accumulation in "muscle" of adrenalectomized rats, but the effect was not statistically significant. Because it might be expected that injected epinephrine would stimulate release of endogenous insulin, the question arises as to why epinephrine injected alone did not produce an "insulin effect" in muscle of adrenalectomized rats. This may be due to the fact that the hyperglycemic response to epinephrine, important for endogenous insulin release, is much reduced in rats following adrenalectomy(4,5).

In regard to the role of corticoids, it appears that long-term treatment of adrenalectomized rats with a glucocorticoid, cortisol, is less effective than is treatment with a mineralocorticoid, DOC, in restoring the ability of muscle to respond to insulin in terms of AIB accumulation against an apparent gradient. These effects of corticoids on AIB transport appear to be indirect, since short-term corticoid treatment did not enhance AIB uptake in adrenalectomized rats, with or without exogenous insulin. Since DOC, and to a lesser extent, cortisol, affect electrolyte metabolism, the findings suggest that maintenance of a suitable ionic balance between cells and plasma may have an important influence upon the process by which AIB is accumulated in muscle, and that the lower accumulation of AIB observed in the adrenalectomized rat after insulin administration may be related to the high plasma K^+ level characteristic of this type animal. This suggestion is in accord with *in vitro* studies, which indicate that high K^+ levels in the medium inhibit glycine transport into isolated diaphragm, and also that ionic shifts are associated with active transport of several amino acids (*cf.* 7). In the present study, plasma $[K^+]$ in adrenalectomized rats (5.85 ± 0.36 μM/ml $n = 18$) is higher than in intact rats (3.74 ± 0.05 μM/ml $n = 4$), or in rats hypophysectomized for 2 days (3.68 ± 0.15, $n = 4$). Administration of

† In regard to results in hypophysectomized rats, it cannot be assumed that residual actions of pituitary hormones, particularly somatotrophin, were entirely absent in 2-day hypophysectomized rats.

epinephrine to adrenalectomized rats, shown to augment muscle AIB accumulation after insulin, significantly reduces the elevated plasma $[K^+]$ of adrenalectomized rats to 4.50 ± 0.28 ($n = 4$), but 6 hr pretreatment with either DOC or cortisol, which had no effect on muscle AIB response to insulin, did not reduce plasma K concentration. Values for other treatments were not obtained, but these preliminary observations indicate that the relationship of plasma $[K^+]$ to the insulin-induced increase in muscle AIB accumulation merits further study.

Independent of the foregoing, the results of this study suggest that epinephrine and corticoids may play a "permissive" or "supportive" role, in the sense proposed by Ingle (8), in the response of muscle to insulin with respect to AIB transport.

Summary. The distribution of AIB *in vivo* in diaphragm and gastrocnemius muscle of intact, hypophysectomized, and adrenalectomized rats has been examined with respect to the effect of insulin and epinephrine and corticoids. Adrenalectomy, but not hypophysectomy, impairs the ability of insulin to increase the accumulation of AIB in muscle to the level observed after insulin in intact rats. Epinephrine alone has slight effect on muscle AIB in adrenalectomized rats, but concurrent administration of epinephrine and insulin to adrenalectomized rats restores AIB distribution nearly to the level observed in adrenal-intact rats receiving insulin. Maintenance treatment of adrenalectomized rats with either DOC or cortisol resulted in normal AIB distributions in diaphragm and both corticoids, especially DOC, also enhance the AIB accumulation response to insulin. The suggestion emerges that these hormonal effects on AIB accumulation may be related to alterations in electrolyte metabolism.

1. Kipnis, D. M., Noall, M. W., *Biochim. et Biophys. Acta,* 1958, v28, 226.

2. Eichhorn, J., Scully, E., Halkerston, I. D. K., Hechter, O., PROC. SOC. EXP. BIOL. AND MED., in press.

3. Eichhorn, J., Halkerston, I. D. K., Feinstein, M., Hechter, O., *ibid.,* 1960, v103, 515.

4. Collip, J. B., Thomson, D. L., Toby, G., *J. Physiol.,* 1936, v88, 191.

5. de Bobo, R. C., Bloch, H. I., Gross, I. H., *Am. J. Physiol.,* 1942, v137, 124.

6. Noall, M. W., Riggs, T. R., Walker, L. M., Christensen, H. N., *Science,* 1957, v126, 1002.

7. Christensen, H. N. *Amino Acid Metabolism,* Ed. W. D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, 1955, p63.

8. Ingle, D. J., *J. Endocrinol.,* 1952, v8, xxxiii.

Received January 10, 1961. P.S.E.B.M., 1961, v106.

Effect of External Cooling of the Leg on Muscle Intracellular Water and Potassium.* (26474)

R. KLEIN AND PATRICIA USHER

Children's Hospital of Pittsburgh and Department of Pediatrics, University of Pittsburgh Medical School

Patients with the hyperkalemic form of familial periodic paralysis become paralyzed upon exposure to a cold environment(1,2). Localized paralysis of the arm has been produced in experiments with these same patients by swathing the forearm in ice packs for ½ hour(3,4). We have postulated that in this disease there is an exaggeration of the normal flux of potassium from the intracellular into the extracellular fluid and an inhibition of the normal return of potassium into the intracellular fluid. Muscle biopsies from one of these patients demonstrated an elevated intracellular water concentration and a depression of intracellular potassium concentration, but no direct evidence was obtained that potassium moved out of the muscle when paralysis was produced by external application of cold. The present studies were carried out to test the hypothesis that intra-

* This work was supported by Grant from Nat. Inst. Health, U.S.P.H.S.