Effects of Epinephrine on the Distribution of Two Model Amino Acids in the Rat

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SUMMARY

Injecting epinephrine into normal fasted rats depressed the serum concentrations of α-aminoisobutyric acid (AIB) and 1-aminocyclopentanecarboxylic acid (ACPC) and simultaneously increased the levels of these model amino acids in liver and heart within 2 hr. The levels of the two compounds in skeletal muscle and diaphragm were unchanged, but the distribution ratios in these tissues were increased because the serum levels decreased. Epinephrine showed a smaller effect at ½ hr.

Injecting the epinephrine antagonist dihydroergotamine methanesulfonate simultaneously with epinephrine removed three-fourths of the increase produced by the hormone in the absolute level and distribution ratio of ACPC in liver, and one-half of the increase in heart. In the presence of the inhibitor, epinephrine was one-third less effective in depressing the serum ACPC level. Neither adrenalectomy nor hypophysectomy greatly diminished the epinephrine-stimulated ACPC transfer into the four tissues examined. The results suggest that the elevated tissue levels found after epinephrine injection are not caused to any large extent by endogenous adrenocortical or hypophyseal hormones, or by insulin.

INTRODUCTION

Epinephrine has long been known to depress the level of amino acids in the blood of a variety of animal species. In 1957, Noall *et al.* (2) demonstrated that the injection of epinephrine into adrenalectomized rats could raise tissue levels of a previously injected dose of the nonmetabolizable amino acid, α-aminoisobutyric acid. They suggested that epinephrine had increased the transport of this amino acid into these tissues. Our studies with insulin (3) suggested that epinephrine secretion was an important factor in bringing about the changes produced by the protein hormone in the distribution of the two model amino acids, α-aminoisobutyric acid and 1-aminocyclopentanecarboxylic acid, in the intact rat. We have therefore examined in more detail the effects of epinephrine on the transport of these amino acids into rat tissues *in vivo*. The results of these experiments are reported here.[2]

MATERIALS AND METHODS

Experiments were carried out in the same manner reported earlier in our studies with insulin (3). Immature female rats[3] (Sprague-Dawley-Holtzman) were used at weights between 80 and 110 g. Adrenalecto-

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[3] Generously supplied by the Upjohn Company, Kalamazoo, Michigan.

mized animals were prepared in this laboratory by surgical removal of the adrenal glands. These animals were maintained on 0.9% NaCl as their drinking water and had an average fasting blood glucose level of 59 mg% at the time they were used. This low value was taken as an index that the adrenalectomy had been successful. Female hypophysectomized rats weighing 80–90 g were obtained from Hormone Assay Laboratories, Chicago, about 10 days after removal of the hypophysis. All animals were maintained on the normal protein test diet purchased from Nutritional Biochemicals Corporation, Cleveland. 1-Aminocyclopentanecarboxylic acid-1-^{14}C (ACPC) and α-aminoisobutyric acid-1-^{14}C (AIB) were used at the same specific activities as reported earlier (3).

The general experimental plan was as follows: Either 0.77 μmole ^{14}C-ACPC or 9.8 μmoles ^{14}C-AIB per kilogram of body weight was injected intraperitoneally into the rats 24 hr before they were sacrificed. All animals were fasted during this period except those that were hypophysectomized, which were continued on the regular diet. Epinephrine was injected subcutaneously at either ½ hr or 2 hr before the animals were killed. When the epinephrine antagonist dihydroergotamine methanesulfonate was used, it was injected intraperitoneally at the same time as the epinephrine. Other details of the procedure and calculations have been given (3).

RESULTS

Effect of Epinephrine in Normal Fasted Rats

Epinephrine injected at a dose level of 1 mg per kilogram body weight 2 hr before sacrifice of the animals led to a nearly 30% decrease in the serum level of either ACPC or AIB (line 1 of Tables 1 and 2). Of the tissues examined, heart and liver showed increases in the absolute levels of both amino acids, the change for ACPC being 2–3 times that for AIB. The changes in skeletal muscle and diaphragm were not significant. The distribution ratios in all four tissues were elevated significantly, however, because of the decreased serum levels (Tables 1 and 2).

When epinephrine was tested at ½ hr it showed a smaller effect than at 2 hr. A dose of 1 mg/kg of the hormone lowered the serum level of ACPC 23% and that of AIB 19% in ½ hr ($P < 0.01$ and <0.02, respectively). The hormone produced hyper-

TABLE 1

Effects of epinephrine on the distribution of 1-aminocyclopentanecarboxylic acid-^{14}C in the immature fasted female rat in 2 hr

Control values were obtained on 5 rats with an average weight of 100 g. The group given 1 mg epinephrine per kilogram contained 10 rats averaging 121 g in weight, that given 0.1 mg/kg had 6 rats averaging 89 g. Final blood glucose levels were: control group, 105 mg%; after 1 mg/kg epinephrine, 182 mg%; after 0.1 mg/kg epinephrine, 109 mg%.

	Tissue level					Distribution ratio				
		% change due to epinephrine					% change due to epinephrine			
Tissue	Control, cpm/μl cell water	1 mg/kg	P	0.1 mg/kg	P	Control	1 mg/kg	P	0.1 mg/kg	P
Serum	1.74 ± 0.05	−27	<0.01	−30	<0.01	—	—	—	—	—
Liver	2.25 ± 0.13	+162	<0.01	−31	<0.02	1.29 ± 0.05	+250	<0.01	−2	NS
Muscle	2.06 ± 0.10	−13	NS[a]	−19	<0.05	1.20 ± 0.05	+11	<0.05	+15	<0.05
Heart	3.32 ± 0.13	+104	<0.01	−26	<0.05	1.91 ± 0.06	+177	<0.01	+4	NS
Diaphragm	3.66 ± 0.30	−1	NS	−7	NS	2.09 ± 0.13	+31	<0.01	+33	<0.01

[a] Difference from control not significant statistically at the 5% level.

TABLE 2

Effect of 1 mg epinephrine per kilogram on the distribution of α-aminoisobutyric acid-^{14}C in the immature female rat in 2 hr

The 10 control rats averaged 85 g in weight and had fasting blood glucose levels of 70 ± 2 mg%. The 7 epinephrine-treated animals averaged 89 g and had fasting blood glucose levels of 148 ± 6 mg%.

	Tissue level, cpm/μl cell H_2O			Distribution ratio		
Tissue	Control	% change due to epinephrine	*P* for change	Control	% change due to epinephrine	*P* for change
Serum	1.57 ± 0.07	−28	<0.01	—	—	—
Liver	10.74 ± 0.39	+48	<0.02	6.32 ± 0.30	+128	<0.01
Muscle	5.86 ± 0.47	+1	NS[a]	3.57 ± 0.25	+48	<0.01
Heart	9.55 ± 1.33	+50	<0.02	5.26 ± 0.53	+144	<0.01
Diaphragm	16.12 ± 0.93	+17	NS	10.40 ± 0.60	+64	<0.01

[a] Difference from control not significant statistically ($P > 0.05$).

glycemia within this time, the blood glucose level having been raised to 168 mg%.None of the tissues showed an increase in the absolute level of either amino acid; although the liver level of AIB was elevated 30%, this increase was not statistically significant with the number of rats tested because of the great variability. The distribution ratio of AIB in the liver was elevated significantly at ½ hr after the hormone, while the distribution ratio of ACPC was increased in heart and diaphragm.

At the longer time of 2 hr, 0.1 mg of epinephrine per kilogram rat was as effective as 1 mg/kg in lowering the serum level of ACPC (a 30% decrease, Table 1), but this dose also resulted in a significant drop in the level of the model amino acid in three of the four tissues (liver, heart, and skeletal muscle; Table 1, column 5). The decreases in ACPC levels in skeletal muscle and diaphragm were less than the drop in the serum level, so that the distribution ratios for these two tissues were increased significantly (Table 1, column 10, lines 3 and 5). These changes were generally of the same qualitative nature as those found for ACPC distribution after 1 mg epinephrine per kilogram had acted for ½ hr. In the latter case, however, the percent decrease in the serum level was greater than the decreases in any of the tissues.

When a total of either 0.1 or 0.2 mg of epinephrine per kilogram was injected in three equal doses over the 2-hr period, none of the changes was qualitatively different from those described above for the 0.1 mg as a single dose. Either dose resulted in a blood glucose level of 140 mg%. This schedule of injections was used because of the possibility that small doses of the hormone might be ineffective over a 2-hr period due to their rapid destruction in the rat.

A 0.25-mg/kg dose of epinephrine altered AIB distribution in ½ hr about as much as did a 1-mg dose in this time; i.e., a significant change was found only in the liver distribution ratio (a 40% increase; $P \ll 0.01$).

Effects of the Blocking Agent, Dihydroergotamine methanesulfonate

Table 3 shows that the rats treated with both 0.3 mg of this inhibitor per kilogram and 1 mg of epinephrine per kilogram for 2 hr had 43% more of the amino acid in their livers and 54% more in their hearts than did those receiving the ergot alkaloid alone. These values compare with the 162% and 104% increases, respectively, found in liver and heart after the same dosage of epinephrine to rats not injected with dihydroergotamine (see lines 2 and 4 of column 3, Table 1). The distribution ratios were affected to the same degree as were the tissue levels. In the alkaloid-treated

TABLE 3

Effect of epinephrine and dihydroergotamine methanesulfonate given together on the distribution of 1-aminocyclopentanecarboxylic acid-^{14}C in the immature female fasted rat

One milligram of epinephrine and/or 0.3 mg dihydroergotamine was injected per kilogram 2 hr before sacrifice. Ten rats were in each group. The animals treated with dihydroergotamine methanesulfonate alone averaged 93 g in weight and had a blood glucose level of 79 ± 4 mg%. Those given the alkaloid drug plus epinephrine weighed 92 g and had an average blood glucose of 130 ± 4 mg%.

	Tissue level, cpm/μl cell H_2O			Distribution ratio		
Tissue	H_2-ergot[a] alone	% change due to epineph-rine	*P* for change	H_2-ergot alone	% change due to epineph-rine	*P* for change
Serum	1.43 ± 0.04	−19	<0.01	—	—	—
Liver	1.91 ± 0.05	+43	<0.01	1.34 ± 0.05	+65	<0.01
Muscle	1.76 ± 0.07	−9	NS[b]	1.21 ± 0.04	+16	<0.02
Heart	2.51 ± 0.08	+54	<0.01	1.75 ± 0.06	+87	<0.01
Diaphragm	3.27 ± 0.05	+10	<0.01	2.27 ± 0.03	+36	<0.01

[a] H_2-ergot = dihydroergotamine methanesulfonate.

[b] Difference from control not significant statistically ($P > 0.05$).

group, the serum level of ACPC was decreased by epinephrine two-thirds as much as it was in the absence of dihydroergotamine (19% *vs* 27%). The hormone-induced changes in diaphragm and skeletal muscle were not influenced by the alkaloid. Dihydroergotamine decreased the distribution ratio of ACPC in the heart 10% in the animals not treated with epinephrine, but it did not alter values in the other tissues (compare the figures in column 7 of Table 1 with those of column 5 of Table 3).

Is the Epinephrine Effect Mediated through Either the Pituitary Gland or the Adrenal Cortex?

Although epinephrine can increase the uptake of ACPC and AIB into the tissues of the intact rat, one cannot tell from the foregoing results whether the effect is direct or whether it is mediated through other hormones, the most likely of which would be those of the adrenal and pituitary glands. It is well known that epinephrine can cause the release of the adrenocorticotropic hormone, which causes adrenal steroid secretion. The glucocorticoids have been shown, in turn, to elevate the uptake of both ACPC (4) and AIB (2, 5–8) by the liver, and to inhibit uptake by muscle tissues (7, 9–11). To study this possibility as well as the possible involvement of other hormones of the pituitary gland, the effects of epinephrine on ACPC distribution were tested in both adrenalectomized and hypophysectomized rats.

Effect of Adrenalectomy

Epinephrine at a level of 1 mg/kg produced an even greater decrease in the serum level of 1-aminocyclopentanecarboxylic acid in adrenalectomized rats than found in intact ones (43 *vs* 27%; Tables 4 and 1). Levels of the amino acid in liver and heart were increased less than in intact rats, presumably because the serum level was lower; but the distribution ratios in all four tissues were elevated nearly as much or more (column 6, Table 4, *vs* column 8, Table 1). This fact suggests that the hormone increased the transport of the amino acid into the tissues to about the same extent in rats with and without their adrenal glands. Less of the ACPC can be accounted for in the adrenalectomized animals, however. Adrenalectomy itself caused only minor changes in ACPC distribution when results were compared with those found in untreated normal rats.

TABLE 4

Effect of epinephrine on the distribution of 1-aminocyclopentanecarboxylic acid-^{14}C in the fasted adrenalectomized rat in 2 hr

Control values were obtained on 15 adrenalectomized rats with an average weight of 110 g. The group given 1 mg of epinephrine per kilogram contained 6 rats averaging 96 g. Final blood glucose levels were: control group 59 ± 3 mg%; after epinephrine 123 ± 8 mg%.

	Tissue level, cpm/μl cell H_2O			Distribution ratio		
Tissue	Control adrex[a]	% change due to epineph-rine	*P* for change	Control adrex	% change due to epineph-rine	*P* for change
Serum	1.72 ± 0.07	−43	<0.01	—	—	—
Liver	2.44 ± 0.04	+52	<0.02	1.50 ± 0.07	+173	<0.01
Muscle	1.91 ± 0.08	−26	<0.02	1.12 ± 0.04	+35	<0.01
Heart	2.81 ± 0.18	+51	<0.01	1.72 ± 0.06	+169	<0.01
Diaphragm	3.78 ± 0.25	+13	NS[b]	2.19 ± 0.09	+113	<0.01

[a] Adrex = adrenalectomized.

[b] Difference from control not significant statistically ($P > 0.05$).

Effect of Hypophysectomy

Removal of the hypophysis likewise did not reduce the ability of epinephrine to increase the uptake of ACPC by the four tissues examined. In these experiments, the rats were fed because fasted hypophysectomized animals were found to accumulate very high levels of injected ACPC in the liver, probably because of endogenous epinephrine secretion in response to the severe hypoglycemia found in fasted rats (see 3). Even the fed hypophysectomized animals had significantly higher liver ACPC levels than did fed intact ones (Table 5).

Serum ACPC levels were the same in fed hypophysectomized animals as in fed intact

TABLE 5

Effect of epinephrine on the distribution of 1-aminocyclopentanecarboxylic acid-^{14}C in fed-hypophysectomized rats and fed-intact rats

Twenty intact-fed rats and 15 hypox[a]-fed rats averaging 88 g in weight were used. Eight hypox and 10 intact rats received 1 mg of epinephrine per kilogram 2 hr before sacrifice. Control animals received saline. Blood glucose values in mg% were: intact fed, 102 ± 7; intact fed given epinephrine, 216 ± 13; fed hypox, 95 ± 6; fed hypox given epinephrine, 238 ± 25.

	Intact fed			Hypox fed		
Tissue	Control	% change due to epinephrine	*P* for change	Control	% change due to epinephrine	*P* for change
Serum, cpm/μl	1.42 ± 0.03	−9	<0.05	1.41 ± 0.01	−9	NS[b]
Liver, D.R.[c]	0.94 ± 0.02	+169	≪0.01	1.36 ± 0.03	+203	≪0.01
Muscle, D.R.	1.41 ± 0.11	−9	NS	1.21 ± 0.02	+16	≪0.01
Heart, D.R.	2.42 ± 0.11	+35	<0.05	2.34 ± 0.10	+42	<0.02
Diaphragm, D.R.	2.45 ± 0.15	+13	NS	2.79 ± 0.10	+34	<0.02

[a] Hypox = hypophysectomized.

[b] Difference from control not significant statistically ($P > 0.05$).

[c] D.R. = distribution ratio of amino acid between tissue water and serum.

animals, and 1 mg/kg epinephrine produced a small decrease in both cases (Table 5). Distribution ratios in the skeletal muscle and diaphragm were elevated by the hormone more in the hypophysectomized rats than in their controls (Table 5), possibly because of the removal of epinephrine-induced ACTH secretion. The latter hormone would normally stimulate adrenocortical steroid release, which in turn could inhibit amino acid uptake by the muscle tissues. Skeletal muscle and diaphragm showed similar increases in sensitivity to epinephrine after adrenalectomy of the rats (compare Table 4 with Table 1). Effects of epinephrine on liver and heart ACPC uptake were essentially the same in hypophysectomized and control animals (Table 5). The results of feeding on the normal and epinephrine-stimulated uptake of ACPC can be seen by comparing the values at the left in Table 5 with those in columns 7 and 8 of Table 1.

DISCUSSION

The most striking effect of epinephrine injection on distribution of the model amino acids was an elevation in their levels in liver and heart that accompanied a drop in the serum levels. No significant changes were found in the levels of either amino acid in the skeletal muscle or the diaphragm. That the transport of ACPC and AIB into these latter two tissues had been increased is shown, however, by the fact that their distribution ratios had been elevated significantly. A drop in the serum level of an amino acid ordinarily results in a corresponding drop in tissue levels within 2 hr, to maintain the original distribution ratio, unless the transport has been changed (see 12).

The results suggest that the changes in amino acid distribution produced by epinephrine were not brought about primarily by adrenocortical or pituitary hormones secreted in response to the epinephrine. Removing the adrenal glands resulted in a greater than normal drop in the serum ACPC level when the hormone was given, but the distribution ratios of all four tissues were elevated to about the same extent, in relation to the serum level, as found in intact rats. Hypophysectomy likewise did not remove any factor that contributed greatly to the elevated uptake.

Secondary secretion of insulin in response to the hyperglycemia may account for part of the change in amino acid distribution found in muscle tissues after epinephrine injection. The protein hormone can increase uptake of both natural and model amino acids by various muscle tissues (3, 8, 13, 14). Nevertheless, the increased uptakes produced by epinephrine in skeletal muscle and diaphragm appear to be at least partly independent of insulin secretion. Distribution ratios in these two tissues were increased significantly when only 0.1 mg of epinephrine per kilogram was administered 2 hr before samples were taken. The blood glucose level was not changed significantly under this condition (it was 105 *vs* 109 mg%, see legend of Table 1), so presumably no endogenous insulin would be released. In contrast, heart muscle could not be shown to increase its ACPC level under any condition of epinephrine dosage that did not elevate the blood glucose level.

Insulin probably does not contribute significantly, however, to the elevated liver levels of the amino acids produced by epinephrine treatment. A relatively large dose of insulin, 0.2 unit/kg, has much less effect on liver ACPC in the absence of the adrenal glands than does 1 mg of epinephrine per kilogram [a 16% increase in distribution ratio (3) *vs* a 173% increase (Table 4)]. Only about 10% of the increase produced by insulin in the intact rat is obtained in the adrenalectomized animal. The difference can be attributed to removal of epinephrine secretion. In addition, epinephrine did not produce changes in liver ACPC under conditions of hyperglycemia which would be expected to produce insulin secretion. For example, blood glucose levels of 168 and 140 mg%, respectively, were found when epinephrine was given for ½ hr, or in 3 small doses over 2 hr. Neither of these treatments produced elevations in liver ACPC. The results with both epinephrine and insulin are best interpreted as showing that the effects of insulin on liver ACPC

levels in intact rats are produced mostly by epinephrine secretion resulting from the insulin hypoglycemia (3).

The changes in tissue amino acid levels cannot be attributed to the hyperglycemia rather than to the epinephrine. In no case was hyperglycemia by itself found to result in an altered amino acid uptake. Epinephrine did not produce increases in muscle tissue levels of AIB or in liver or skeletal muscle levels of ACPC within ½ hr, even though the blood glucose had been elevated to 168 mg% in these rats. In addition, for the liver the large increase in amino acid content seen after insulin injection is probably a result of epinephrine secretion (3). Hypoglycemia, rather than hyperglycemia, was present in these rats.

Since the evidence suggests that epinephrine acts directly on the liver to alter amino acid transport, we may conclude that dihydroergotamine acts directly as an inhibitor of this effect of epinephrine. Skeletal muscle and diaphragm did not respond to the alkaloid, perhaps because of their relatively low sensitivity to epinephrine. The large effect on the heart is probably also produced by a direct blocking action, even though the data do not show this conclusively.

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