The Influence of Corticosteroids on Deamination in the Surviving Diaphragm

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The surviving isolated diaphragm of the rat takes up glucose and produces glycogen. These processes are inhibited by deoxycorticosterone (Verzár & Wenner, 1948*a, b*) and also by 11-dehydro-17-hydroxycorticosterone (Leupin & Verzár, 1950). It seemed possible that changes in the protein metabolism under the influence of the added corticosteroids might lead to a change in the rate of deamination of amino-acids. The ammonia production in the diaphragm of normal pithed rats in the presence and absence of corticosteroids was therefore studied.

METHODS

The animal was killed and the diaphragm divided into two equal parts; these were suspended in 2 ml. of phosphate-

buffered oxygenated Ringer solution at 37° with the addition of 200 mg. glucose/100 ml. of fluid and 1 i.u. insulin/100 ml. as in earlier experiments. One half of the diaphragm was used without addition and the other with the addition of a corticosteroid. In other experiments a different corticosteroid was added to each half of the muscle. All additions were made in ethanolic solution as previously described (Leupin & Verzár, 1950).

Measurement of ammonia production. After 90 min. incubation the NH_3 content was estimated by the method of Conway & O'Malley (1942) and Conway (1947). The solution (0.5 ml.) was deproteinized by Folin's method and then transferred to the outer chamber of a Conway vessel together with 1 ml. of saturated K_2CO_3 . The inner chamber of the Conway vessel contained 1 ml. of 1% (w/v) boric acid in 20% (v/v) ethanol with bromocresol green-methyl red indicator. The vessels were greased with a hard fat instead of

Table 1. *Ammonia production by diaphragm with and without the addition of various corticosteroids*

Exp. no.	Diaphragm without addition of corticosteroid. NH_3 produced (mg./100 g. of muscle)	Diaphragm with addition of corticosteroid	
		Compound added (mg./100 ml.)	NH_3 produced (mg./100 g. of muscle)
1	8.1	DOC, 4.4	14.7
2	4.3	DOC, 4.4	4.7
3	22.5	DOC, 5	7.4
4	6.1	DOC, 5	7.4
5	5.8	DOC, 5	7.3
6	6.5	DOC, 5	4.7
7	6.1	DOC, 5	7.3
8	18.0	DOC, 5	10.9
9	10.4	DOC, 5	19.7
10	9.4	DOC, 5	10.4
11	7.6	DOC, 5	8.2
12	10.0	DOC, 5	11.9
13	7.8	DOC, 5	8.2
14	7.4	DOC, 5	8.0
15	2.9	DOC, 5	9.6
16	12.6	DOC, 5	11.8
17	6.1	DOC, 5	6.3
18	12.0	DOC, 5	7.9
19	9.0	DOC, 5	10.1
20	7.7	DOC, 5	7.7
21	10.3	DOC, 5	8.1
22	3.7	DOC, 7	3.9
23	6.6	DOC, 7	4.3
24	4.0	DOCA, 5	14.1
25	7.2	DOCA, 5.6	6.2
26	9.6	DOCA, 5.6	11.3
27	11.5	DOCA, 5.6	10.6
28	4.0	DOCA, 5.6	10.2
29	8.9	DOCA, 5.6	6.7
30	7.2	Compound E, 5	7.2
31	10.7	Compound E, 5	14.0
32	8.3	Compound E, 5	15.1
Mean	8.5 ± 2.2		9.2 ± 2.0

Table 1 (cont.)

In most of the following experiments the effects of two different corticosteroids on the two halves of the same diaphragm were compared:

Exp. no.	Diaphragm with addition of corticosteroid		Diaphragm with addition of corticosteroid	
	Compound added (mg./100 ml.)	NH ₃ produced (mg./100 g. of muscle)	Compound added (mg./100 ml.)	NH ₃ produced (mg./100 g. of muscle)
33	DOCA, 5	8.4	E, 5	8.7
34	DOCA, 5	17.2	E, 5	11.6
35	DOCA, 5	7.2	E, 5	6.3
36	DOC, 5	12.2	E ₁ , 5	21.8
37	DOC, 5	10.5	E ₁ , 5	10.3
38	DOC, 5	11.9	E ₁ , 5	28.0
39	DOC, 5	6.5	E ₁ , 5	8.9
40	DOC, 5	9.3	E ₁ , 5	9.3
41	None	13.3	E ₁ , 10	9.8
42	None	9.9	E ₂ , 5	5.6
43	E ₁ , 5	25.0	E ₂ , 5	10.6
44	DOC, 5	10.8	E ₂ , 5	10.0
45	None	9.0	E ₁ + E ₂ , 15	5.6
46	DOC, 5	8.0	E ₁ + E ₂ , 15	8.3
47	DOC, 5	7.8	E ₁ + E ₂ , 15	9.7
48	E ₁ , 10	8.7	DOC, 5	11.5
	Mean	10.9 ± 0.3		11.0 ± 2.1

vaseline. The reaction time was 4 hr. Titration was with 0.002 or 0.001 N-HCl from a horizontal Conway burette. The NH₃ values in Table 1 are given as mg. NH₃/100 g. muscle.

RESULTS

Table 1 shows the results of thirty-two experiments in which ammonia production was measured with and without addition of corticosteroids, and also of sixteen experiments in which different corticosteroids were applied to the two halves of the muscle. DOC represents deoxycorticosterone and DOCA its acetate. Compound E is the acetate of 11-dehydro-17-hydroxycorticosterone; E₁ is the crystalline fraction and E₂ the amorphous residue, obtained by saponifying compound E. E₁ + E₂ is a mixture of E₁ and E₂ in the proportion 1:2 (Leupin & Verzár, 1950).

The mean value of ammonia production was 8.5 mg. NH₃/100 g. muscle in experiments in which no addition was made to the Ringer solution; the corresponding value in experiments in which DOC (4.4–7.0 mg./100 ml.) or DOCA and compound E (in similar concentrations) were added was 9.2 mg. The mean values for the experiments in which different corticosteroids were added to two halves of the same muscle were 10.9 and 11.0 mg. NH₃/100 g. muscle respectively. Values for the two halves of the same muscle generally agreed fairly well as shown in the

second series and no significant effects of the different corticosteroids on ammonia production were observed. However, ammonia figures for different muscles even without addition of steroids showed large individual variations and extreme values as low as 3.7 mg. and as high as 28 mg./100 g. muscle were observed.

DISCUSSION

No effect of the addition of various corticosteroids on the ammonia production by isolated muscle has been observed. The inhibition of glycogen production from glucose does not involve an increased deamination of amino-acids. This negative result with isolated surviving muscle must of course not be applied to the intact animal.

SUMMARY

1. The surviving diaphragm of the rat incubated in oxygenated Ringer solution containing 200 mg. glucose and 1 i.u. insulin/100 ml. produces about 8.5 ± 2.2 mg. of ammonia/100 g. muscle in 90 min.
2. Addition of deoxycorticosterone and 11-dehydro-17-hydroxycorticosterone did not alter the ammonia production.

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