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EFFECTS OF PITUITARY GROWTH HORMONE AND CORTICOTROPIN ON FAT METABOLISM IN ISOLATED RAT DIAPHRAGM

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SUMMARY

The effect of hypophysectomy and of some pituitary hormones *in vitro* on the incorporation of ^{14}C from acetate and palmitate into lipid and protein of isolated rat diaphragm has been studied. The most striking effects observed were: a diminution of incorporation into lipid as a result of hypophysectomy, a reduction in incorporation into lipid by growth hormone and corticotropin in diaphragm from normal animals, but not by growth hormone in diaphragm from hypophysectomized animals, and a stimulation of incorporation into protein by both hormones in diaphragm from hypophysectomized animals.

INTRODUCTION

Although the extent of the actions *in vitro* of pituitary growth hormone on the metabolic activity of isolated diaphragm from normal rats is confused[1-3], the hormone undoubtedly affects the metabolism of diaphragm from hypophysectomized animals[4-6]. These actions have been called "insulin-like" actions since they are similar to the action of insulin and not always consistent with the action *in vivo* of growth hormone. OTTAWAY[7] suggested that the insulin-like actions resulted from an action of growth hormone to release insulin bound in tissues in an inactive form.

Insulin has recently been shown to stimulate incorporation *in vitro* of ^{14}C from acetate into fat and phospholipid of diaphragm and of ^{32}P into phospholipid and protein[8]. In view of the well established lipolytic activity of pituitary growth hormone it was thought of particular interest to determine whether the hormone would also stimulate these processes in diaphragm from hypophysectomized rats. This was not found to be the case, but in the course of the investigation effects of growth hormone and of corticotropin on fat synthesis in normal diaphragm were noticed and the purpose of this communication is to record these observations.

METHODS

Hemidiaphragms, from non-fasting female albino Wistar rats, 100–120 g, were incubated for 2 h at 37° in Krebs bicarbonate buffer (pH 7.4), gassed with O_2–CO_2 (95:5). The buffer contained no glucose. Radioisotopes were obtained from the Radiochemical Centre, Amersham (Great Britain). Sodium [1-^{14}C]acetate, specific activity

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about 4.5 μC/μmole, was added at a concentration of 0.8 μC/ml (0.2 μmole/ml). [$^{14}C_{16}$]Palmitic acid, specific activity about 76 μC/μmole, was supplied as a solution in benzene. The solution was evaporated to dryness and the residue dissolved in a small quantity of ethanol which was diluted with buffer to a concentration of 0.4 μC/ml (5 mμmoles/ml). [^{32}P]Phosphate was present with activity of about 4 μC/ml. Since the concentration of phosphate was 1.2 mM its specific activity was about 3.3 μC/μmole. Growth hormone was prepared in this laboratory by Mr. B. R. SLATER from ox pituitary lobes by the method of WILHELMI, FISHMAN AND RUSSELL[9]. The same preparation (66GHI) was used for the work of Tables I and III. The growth hormone used in Table IV was another sample (73GH3) and the highly purified material was this sample chromatographed on DEAE-cellulose[10] by Mr. M. WALLIS. Corticotropin A_1 (ref. 11) was kindly provided by Dr. H. B. F. DIXON.

The technique for preparation of lipid samples was based on methods described by NEPTUNE *et al.*[12] and FOLCH *et al.*[13] and has been previously described[8]. Samples of protein were prepared as described by MANCHESTER[14]. In the latter stages of the work analysis of lipid fractions labelled with ^{14}C from acetate was carried out by thin-layer chromatography[15]. The bulk (70%) of the radioactivity was found in triglyceride, the remainder being distributed between fatty acids, cholesterol and various phospholipids.

RESULTS

Incorporation of ^{32}P into phospholipid and protein

Neither hypophysectomy nor addition of growth hormone *in vitro* to diaphragm from normal or hypophysectomized rats were found to influence the incorporation of [^{32}P]phosphate into phospholipid or protein (Table I). This parameter was therefore not investigated further.

TABLE I

EFFECT OF HYPOPHYSECTOMY AND OF ADDITION *in vitro* OF GROWTH HORMONE ON THE INCORPORATION OF ^{32}P FROM [^{32}P]PHOSPHATE INTO PHOSPHOLIPID AND PROTEIN OF ISOLATED RAT DIAPHRAGM

Each figure is the mean ± S.E. of the mean of 12 observations.

Fraction in which radioactivity is contained after incubation	*Radioactivity in diaphragm from*				*Significance of differences*
	Normal rats		*Hypophysectomized rats*		
	Additions		*Additions*		
	No addition	*Growth hormones*	*No addition*	*Growth hormone*	
Phospholipid (counts/min per g wet wt. of diaphragm	29 600 ± 2000	27 400 ± 1000	29 300 ± 1500	29 500 ± 2300	—
Protein (counts/min per mg of protein)	119 ± 10	124 ± 11	123 ± 11	131 ± 13	—

Incorporation of ^{14}C from acetate and palmitate into lipid and protein

Effect of hypophysectomy: Hypophysectomy decreased the incorporation of ^{14}C from acetate into neutral fat, phospholipid and protein irrespective of whether the rats were fed *ad libitum* (Table II) or when the food intake of the normal was limited to that of the hypophysectomized animals (Table III). Incorporation of ^{14}C into

TABLE II

EFFECT OF HYPOPHYSECTOMY AND OF ADDITION *in vitro* OF PURIFIED GROWTH HORMONE ON THE INCORPORATION OF ^{14}C FROM [1-^{14}C]ACETATE AND [$^{14}C_{16}$]PALMITATE INTO NEUTRAL FAT, PHOSPHOLIPID AND PROTEIN OF ISOLATED RAT DIAPHRAGM

Each figure is the mean ± S. E. of the mean of 12 observations.

Fraction in which radioactivity is contained after incubation with	*Radioactivity in diaphragm from*				*Significance of differences (P)*
	Normal rats		*Hypophysectomized rats*		
	Additions		*Additions*		
	No addition (a)	*Growth hormone (b)*	*No addition (c)*	*Growth hormone (d)*	
[1-^{14}C]Acetate:					
Neutral fat (counts/min per g wet wt. of diaphragm)	16 500 ± 2000	11 400 ± 1200	9300 ± 1200	10 300 ± 200	(a—b) < 0.05 (a—c) < 0.01
Phospholipid (counts/min per g wet wt. of diaphragm)	3380 ± 200	2790 ± 160	2670 ± 100	2650 ± 130	(a—b) < 0.05 (a—c) < 0.01
Protein (counts/min per mg of protein)	320 ± 19	280 ± 12	230 ± 20	290 ± 26	(a—c) < 0.01
[$^{14}C_{16}$]Palmitate:					
Neutral fat (counts/min per g wet wt. of diaphragm)	140 000 ± 5200	144 000 ± 5400	120 000 ± 5600	135 000 ± 7300	(a—c) < 0.02
Phospholipid (counts/min per g wet wt. of diaphragm)	51 100 ± 1600	52 500 ± 2600	48 400 ± 2800	52 100 ± 2200	
Protein (counts/min per mg of protein)	39 ± 2.9	38 ± 3.2	41 ± 2.0	53 ± 3.5	(d—c) < 0.01

protein also was less in the hypophysectomized animals (Tables II and III). In addition hypophysectomy depressed incorporation of ^{14}C from palmitate into fat (Table II).

TABLE III

EFFECT OF HYPOPHYSECTOMY ON THE INCORPORATION OF ^{14}C FROM [1-^{14}C]ACETATE INTO NEUTRAL FAT, PHOSPHOLIPID AND PROTEIN OF ISOLATED RAT DIAPHRAGM (FOOD INTAKE OF NORMALS LIMITED TO THAT OF HYPOPHYSECTOMIZED RATS)

Each figure is the mean ± S. E. of the mean of 12 observations.

Fraction in which radioactivity is contained	*Radioactivity in diaphragm from*		*Significance of differences (P)*
	Normal rats	*Hypophysectomized rats*	
Neutral fat (counts/min per g wet wt. of diaphragm)	17 500 ± 920	8700 ± 400	< 0.001
Phospholipid (counts/min per g wet wt. of diaphragm)	1800 ± 280	1260 ± 100	< 0.1
Protein (counts/min per mg protein)	172 ± 5.4	155 ± 3.1	< 0.02

Addition of growth hormone: Addition of growth hormone to the medium (50 μg per ml) diminished incorporation of ^{14}C from acetate into lipid but not into protein (Table II) in diaphragm from normal rats. A similar action was not seen with diaphragm from hypophysectomized rats—the hormone did not reduce incorporation of ^{14}C from acetate into fat and in the case of protein the S.E. of the mean of the paired differences (52 ± 18) was such that the P value for the stimulation produced by growth hormone was < 0.02. The lowest concentration at which an action of growth hormone could be consistently detected was about 5 μg/ml (Table IV).

By contrast with the results obtained with acetate, addition of growth hormone did not influence incorporation of ^{14}C from palmitate into fat or phospholipid in diaphragm from normal rats or from hypophysectomized animals (Table II). Growth hormone did, however, stimulate incorporation of ^{14}C from palmitate into protein of diaphragm from hypophysectomized rats (Table II).

Effect of corticotropin: Corticotropin *in vitro* inhibited incorporation of ^{14}C from acetate into fat and phospholipid of diaphragm from normal animals, the minimum active concentration being about 1 μg/ml (Table IV). At 0.1 μg/ml no activity was observed. Like growth hormone, it did not significantly inhibit incorporation of ^{14}C into protein. Unlike growth hormone it inhibited incorporation of ^{14}C from acetate into fat in diaphragm from hypophysectomized rats as well as from normal animals (Table IV). Like growth hormone it raised incorporation of ^{14}C into protein in this preparation (Table IV). However, whereas growth hormone *in vitro* will raise incorporation of amino acids into protein in diaphragm from the hypophysectomized rat, corticotropin does not (unpublished observations).

DISCUSSION

Hypophysectomy

The diminution of incorporation into fat and phospholipid of ^{14}C from acetate but not of ^{14}C from palmitate or of ^{32}P from phosphate suggests that hypophysectomy

TABLE IV

EFFECT OF GROWTH HORMONE AND CORTICOTROPIN ON THE INCORPORATION OF ^{14}C FROM [1-^{14}C]ACETATE INTO NEUTRAL FAT, PHOSPHOLIPID AND PROTEIN OF ISOLATED RAT DIAPHRAGM

Each figure is the mean ± S.E. of the mean of the number of observations shown in parentheses. The value of P for a difference which is significant is indicated. The growth hormone used in this table had been purified by fractionation on DEAE-cellulose[10].

Additions to the medium	*Neutral fat (counts/min per g wet wt. of diaphragm)*			*Phospholipid (counts/min per g wet wt. of diaphragm)*			*Protein (counts/min per mg of protein)*		
	Control	*Experiment*	*Difference*	*Control*	*Experiment*	*Difference*	*Control*	*Experiment*	*Difference*
Purified growth hormone (12) (50 μg/ml)	11 800 ± 1480	7650 ± 890 $P < 0.05$	4150 ± 1250 $P < 0.01$	3510 ± 460	3100 ± 320	410 ± 390	460 ± 28	483 ± 37	23 ± 43
Purified growth hormone (6) (5 μg/ml)	16 300 ± 830	10 800 ± 1250 $P < 0.01$	5580 ± 1130 $P < 0.01$	4330 ± 363	4130 ± 330	200 ± 418	336 ± 18	287 ± 14 $P = 0.05$	48 ± 26
Corticotropin A_1 (6) (50 μg/ml)	18 300 ± 3100	8400 ± 1400 $P < 0.02$	9900 ± 2300 $P < 0.02$	3610 ± 390	2585 ± 275 $P = 0.05$	1025 ± 83	410 ± 28	386 ± 20	24 ± 17
Corticotropin A_1 (6) (1 μg/ml)	12 800 ± 1910	8100 ± 934 $P = 0.05$	4700 ± 2400	2390 ± 130	2020 ± 155	370 ± 130 $P < 0.05$	224 ± 15	210 ± 15	14 ± 19
Corticotropin* (9) (50 μg/ml)	15 400 ± 1800	8700 ± 900 $P < 0.01$	6700 ± 1500 $P < 0.01$	2830 ± 400	2520 ± 390	310 ± 210	263** ± 16	318** ± 15 $P = 0.02$	55** ± 13 $P < 0.01$

* Diaphragm from hypophysectomized rats.
** 12 observations.

affects primarily the conversion of acetate to longer-chain fatty acids rather than the incorporation of the latter into lipids. A reduced capacity to take up acetate or to transform it to acetyl-CoA (though a normal capacity to form palmityl-CoA) would be a possible explanation and would be consistent with the reduction of the incorporation into protein of ^{14}C from acetate but not from palmitate.

Growth hormone

The insulin-like activity of growth hormone looked for in lipid metabolism of diaphragm from hypophysectomized rats was not found, but in its place an inhibitory action of the hormone on incorporation of ^{14}C from acetate into diaphragm from normal animals was observed. Why the inhibitory action is not also seen with diaphragm from hypophysectomized animals, as is the case with corticotropin (Table IV), is not clear, but it is possible that under these circumstances the hormone has a double action—an insulin-like stimulation and an inhibitory effect as with normal diaphragm—resulting in no observable change. In the case of incorporation of ^{14}C from palmitate into protein, where the inhibitory effect is lacking with normal diaphragm, the stimulation with the hypophysectomized diaphragm is quite pronounced.

Since with diaphragm from the normal animals growth hormone does not influence the incorporation into any fraction of ^{14}C from palmitate or of ^{32}P but only when the radioactivity is derived from acetate, it appears that the hormone is affecting, primarily, as with hypophysectomy, the conversion of acetate to longer-chain fatty acids or to a less extent to amino acids. However, since growth hormone *in vitro* can also promote the release of glycerol by isolated diaphragm[16], an influence on esterification of fatty acids would provide another point at which regulation could be exerted.

Corticotropin

The inhibitory activity of corticotropin on incorporation of ^{14}C from acetate into lipid clearly adds to the list of extra-adrenal actions of this hormone[17]. It also raises the question of whether the activity of growth hormone is in reality due to contamination with corticotropin. However, if the minimum level of activity of the latter were as low as 0.1 μg/ml, growth hormone would have to contain at least 2% of corticotropin for its activity to be the result of contamination. The upper limit of the amount of corticotropin in growth hormone prepared by the WILHELMI method is about 0.05% (ref. 9) and that of growth hormone further purified by chromatography on DEAE-cellulose, which retained inhibitory activity (Table IV), must be even lower. The capacity of corticotropin to enhance incorporation of ^{14}C from acetate into protein of diaphragm from hypophysectomized rats is reminiscent of the "insulin-like" activity of growth hormone, but that this activity should not also occur for incorporation of amino acids remains a curious and inexplicable observation.

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