THE EFFECT OF STARVATION ON TISSUE ADENOSINE 3'-5' MONOPHOSPHATE LEVELS

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SUMMARY

The effect of starvation on intracellular levels of 3'-5', cAMP in liver, kidney, muscle, fat and isolated islets of Langerhans was studied. It was found that the concentration of cAMP increased in liver, kidney, muscle and fat, but decreased in the islets after 48-72 hours of starvation. Similar studies were conducted on liver, kidney, muscle and fat of hypophysectomized rats and identical results were obtained.

INTRODUCTION

The intracellular level of 3'-5' cyclic AMP is an important controlling factor in many cellular processes and has been shown to be the key intermediate for the action of peptide hormones such as glucagon and epinephrine on glycolysis and lipolysis (1) (2). The cyclic nucleotide is also one of three key signaling factors required for insulin release from the β cells of the islets of Langerhans (3). The other two factors are calcium ion (4) and glucose and/or a metabolite of glucose (5). The role which cAMP fulfills in this regard is obscure, though it has been postulated that the cyclic nucleotide might be required for calcium translocation within the β cell (6). Little is known about intracellular cAMP levels and fluctuations in diseased states. This study was undertaken to examine cAMP levels in tissues concerned with carbohydrate metabolism after a period of starvation. Starvation was chosen as a model of the diabetic state in rats since metabolic changes which occur after a period of fasting are characteristic also of those described in diabetes mellitus. These changes include increased lipolysis and

glycolysis (7), increased gluconeogenesis (8), increased peripheral insulin resistance and decreased insulin release upon a glucose stimulus (9) (10).

METHODS

Male Sprague-Dawley rats weighing between 150-200 g were used in all the studies. They were permitted free access to a standard diet except during fasting when their intake was restricted to water alone. Tissue analyses on fasted animals were conducted on normal rats after they were fasted for 48 to 72 hours and on hypophysectomized rats after 24 hours of fasting. Hypophysectomized rats were studied 3 to 4 weeks postoperatively.

For the preparation of tissues for DNA and cAMP analyses, the methods of Hofert and Gilman were followed respectively (11) (12). After induction of anesthesia with nembutal (0.1 mg/100 gms), the rats were allowed to sleep for at least one half hour before the tissues were removed. Small pieces of abdominal muscle, epididymal fat pad, liver and kidney cortex were rapidly removed, in that order, and immediately frozen in liquid nitrogen. The frozen tissue samples were weighed and then homogenized in a small aliquot of ice cold 10% TCA in a motor driven homogenizer. The precipitate was removed by centrifugation at 3,000 rpm for 10 minutes and processed for DNA analyses as outlined by Hofert (11). A DNA determination was carried out on each piece of tissue studied. The indole method as described by Hubbard (13) was used.

The supernatant fraction was collected and treated for cAMP analyses. One N HCL was added to give a final concentration of 0.1 N before it was extracted 4 times with 2 volumes of ether. Any excess ether was boiled off in a waterbath at 90° for 3 minutes. The extract was lyophylized overnight and after the addition of a small volume of 50 mM NaAc pH 4 to the dried precipitate, cAMP determinations were done by the Gilman method (12).

TABLE I

THE EFFECT OF STARVATION ON THE RATIO $\frac{\text{DNA}}{\text{mg. wet weight}}$ IN NORMAL AND HYPOPHYSECTOMIZED RATS.

	CONCENTRATION OF DNA µg/mg. WET WEIGHT			
	LIVER	KIDNEY	MUSCLE	FAT
NORMAL:				
FED	2.14 ± .11	3.33 ± .09	0.58 ± .005	0.16 ± .006
FASTED 48hr	3.98 ± .09	3.85 ± .1	0.65 ± .04	0.24 ± .03
72hr	4.20 ± .4	5.34 ± .19	0.90 ± .04	0.33 ± .02
HYPOPHY-SECTOMIZED:				
FED	3.40 ± .24	7.43 ± .18	1.18 ± .07	0.48 ± .03
FASTED 24hr	4.48 ± .17	7.95 ± .32	1.28 ± .06	0.76 ± .18

Values are means and S.E.M. of 15 determinations

Islets of Langerhans were isolated following collagenase treatment of the whole pancreas as outlined by Lacy (14). Groups of 50 to 100 islets were collected and homogenized in a small aliquot of 10% TCA and similar steps as outlined before were followed for DNA and cAMP determinations on the homogenate. A cAMP assay was also conducted on the Hank's solution in which the islets were suspended. Results given are the sum of the two values.

RESULTS AND DISCUSSION

DNA determinations performed on tissues of fed and fasted normal and hypophysectomized rats are summarized in Table I. Starvation was accompanied by an increase in the ratio µg DNA/mg wet weight in all tissues studied. In liver, kidney, muscle and fat, DNA levels rose progressively with duration of fasting and reached a maximum 72 hours after the onset of starvation. A significant increase in DNA per mg wet weight was found in tissues of hypophysectomized rats when compared to the fed normal rats, and a further rise occurred in these tissues

TABLE II
3'-5' cyclic AMP LEVELS IN TISSUES
FROM FED AND FASTED NORMAL RATS.

	3'-5' cyclic AMP CONCENTRATION PICOMOLES/mg DNA	
	FED NORMAL	FASTED (48-72hr) NORMAL
LIVER	343 ± 14	581 ± 104
KIDNEY	597 ± 49	947 ± 299
MUSCLE	760 ± 123	1301 ± 246
FAT	535 ± 48	767 ± 181

Values are means and S.E.M. of 10 determinations

with fasting. Since the concentration of DNA per cell probably remains constant, these results suggested that starvation caused a change in cell density per unit weight in the tissues. Our results per unit wet weight were comparable to values reported by other investigators, but since we observed the altered ratio µg DNA/mg wet weight, this had to be taken into consideration for an accurate expression of the concentration of cAMP in tissues following starvation. All results were thus expressed as picomoles per mg DNA.

Table II illustrates the effect of starvation on tissue cAMP concentrations in liver, kidney, muscle and fat. The values determined for tissues of fasted rats were higher than the corresponding values for tissues of fed litter mates. These results are in accord with those of Jefferson (15) and Freedland (16) who reported a rise in the liver cAMP level of rats after a period of fasting. Evidence exists that the accumulation of cAMP in tissues of fasted rats occurs in response to increased concentrations of glucagon and epinephrine and decreased levels of insulin (17). Of these hormones, glucagon stimulates adenylcyclase in liver (1) and fat (2), and epinephrine effects the same enzyme in liver (1), fat (18), muscle (19) and kidney (20) with a resultant increased synthesis of cAMP. Pauk (21) also demonstrated an

TABLE III
3'-5' cyclic AMP CONCENTRATION IN TISSUES FROM FED AND FASTED HYPOPHYSECTOMIZED RATS

	3'-5' cAMP CONCENTRATION PICOMOLES/mg DNA	
	FED HYPOPHYSECTOMIZED	24 hr FASTED HYPOPHYSECTOMIZED
LIVER	100 ± 15	510 ± 174
KIDNEY	247 ± 90	1062 ± 422
MUSCLE	1052 ± 429	2083 ± 418
FAT	438 ± 46	658 ± 41

Values are means and S.E.M. of 10 determinations

enhanced sensitivity of the liver and fat adenylcyclase enzyme to glucagon action in rats fasted 18-24 hours when compared to the sensitivity of the enzyme to glucagon action in the tissues of fed rats.

An analysis of the cAMP response to starvation in the tissues of hypophysectomized rats is shown in Table III. The values determined for tissues of rats fasted for 24 hours were higher than the comparable values for tissues of fed rats. These results suggest that the cAMP response to glucagon stimulation is not impaired in rats lacking pituitary hormones even though the gluconeogenic and lipolytic responses to an identical hormonal stimulus are absent (22). These results are similar to those of Exton (22) who reported an absent gluconeogenic response to glucagon in adrenalectomized animals which could be restored to normal with the injection of dexamethasone to these animals. They showed that the perfusion of livers of adrenalectomized rats with glucagon resulted in an increase in cAMP levels (22).

The results of cAMP determinations on isolated islets of Langerhans of fed and fasted rats are summarized in Table IV. In sharp contrast to the findings reported for other tissues, the cAMP concentrations decreased significantly in islets obtained from fasted rats. Several

TABLE IV
EFFECT OF STARVATION ON 3'-5' cAMP LEVELS
IN ISOLATED RAT ISLETS OF LANGERHANS

3'-5' cAMP CONCENTRATION (PICOMOLES/MG DNA)	
FED	FASTED 48-72 HR
1999 ± 789	640 ± 176

Determinations on 1000 fed islets and 500 starved islets.

investigators have reported that glucose-induced insulin release is impaired in animals after a period of starvation (10) (23). Since insulin release could be restored to normal in an in vitro system with agents such as theophylline and tolbutamide which are known to increase intracellular levels of cAMP, it was postulated by Voyles and co-workers (10) that the impairment of the glucose-mediated insulin release from islets of fasted rats might be a decreased level of cAMP. The observation reported here, however, has shown for the first time a definite link between a decreased level of a known factor, i.e., cAMP, and the defective insulin response observed in the fasted state. A drop in the cAMP level in the islets of Langerhans after starvation could be attributed to increased phosphodiesterase activity, a decreased adenyl-cyclase activity or a β cell response to the increased catecholamine release which takes place during starvation. Epinephrine stimulation causes a drop in cAMP levels in isolated islets of Langerhans as has been shown by Turtle and co-workers (24). These possibilities are presently being explored in this laboratory.

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