# HORMONE STIMULATION OF KETOGENESIS IN RAT LIVER HOMOGENATES

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Summary:

Cyclic 3',5'-AMP and theophylline were found to stimulate ketogenesis in rat liver homogenates. Data is presented on the effect of various cofactors on ketone body production. In appropriately fortified homogenates glucagon, cyclic AMP and theophylline were found to increase acyl CoA levels and stimulate ketogenesis.

Recent reports from several laboratories have indicated that both the gluconeogenic and ketogenic effects of glucagon are mediated by cyclic 3',5'-AMP (1-5). It has been proposed (1, 5-6) that these effects produced by glucagon are initiated by the activation of a hepatic lipase, thereby increasing the intracellular levels of free fatty acids (FFA). FFA in turn have been shown to stimulate hepatic glucose and ketone body production (6-8). Further support for this mechanism has come from experiments showing that glucagon, cyclic AMP and theophylline stimulate FFA release from rat liver slices and also increase tissue levels of coenzyme A esters (4, 9-10). McCraw *et al.* (11), have recently reported that the addition of triglycerides to isolated perfused livers would stimulate glucose and ketone body production. This elevated gluconeogenesis and ketogenesis could be further increased by the addition of glucagon or theophylline. The present communication reports experiments on hormone stimulation of ketogenesis in rat liver homogenates.

## Materials and Methods:

Male albino rats (240-300 g) of the Cox-Holtzman strain maintained on water and Purina Laboratory Chow *ad libitum* were fasted 16-18 hours

prior to use and then anesthetized by an intraperitoneal injection of sodium amobarbital (125 mg/kg). The liver was removed, washed and chilled. Homogenates were prepared using the lowest speed of a Polytron homogenizer[a] (10-15 sec.) in calcium free Krebs-Ringer Phosphate buffer (pH 7.4). Each incubation flask contained 2.5 ml of 20% homogenate. Glucagon (Lot No. 258-234B-167-1; kindly supplied by Eli Lilly and Co.), cyclic 3',5'-AMP (Sigma Chemical Co.,) or cofactors were dissolved in a small volume of 0.154 M NaCl and added to the flasks. An equal volume of NaCl was added to control flasks. Theophylline was dissolved in buffer and added to concentrated homogenates to bring the final homogenate concentration to 20%. Incubations were for 90 min. at 37° in a shaking water-bath. The reaction was stopped by the addition of perchloric acid (final concentration was 6% v/v). The extracts were placed in ice for 20 min., centrifuged, neutralized with 5 M $K_2CO_3$, chilled and centrifuged again. Ketones were estimated enzymatically in the supernatant by the fluorometeric[b] method of Young and Renold (12). The long-chain acyl CoA esters were determined in the perchloric acid insoluble precipitate (9).

Results:

The effect of glucagon cyclic AMP and theophylline on total ketone body production (acetoacetate + β-hydroxybutyrate) by rat liver homogenates is shown in Fig. 1. Little stimulation was observed with glucagon; however, 3',5'-AMP was found to significantly stimulate ketogenesis ($p < .05$). The greatest response was seen with theophylline ($p < .01$). Theophylline presumably exerts its action by elevating the tissue levels of cyclic AMP via inhibition of cyclic nucleotide phosphodiesterase, the enzyme responsible for the catabolism of cyclic AMP. In order to obtain a system

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[a] Willems Polytron Brinkman Instruments Inc.

[b] Fluorometric measurements were made using the Aminco Fluoromicrophotometer, American Instrument Co.

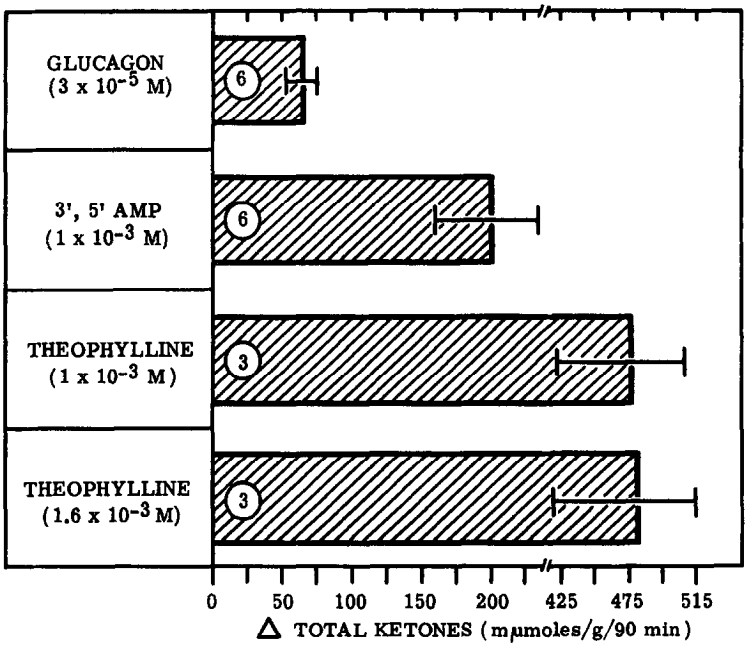


Fig. 1. Effect of glucagon, cyclic AMP and theophylline on ketogenesis in rat liver homogenates. Conditions of incubation were the same as those described in materials and methods. Values represent the mean change from control ± SE of the mean in paired homogenates. Values in circles represent the number of animals used.

in which fatty acid activation and oxidation to ketones would not be rate-limiting, various cofactors were added to liver homogenates and ketone body production was followed (Table 1). The greatest increase in ketogenesis was seen when carnitine and α-ketoglutarate were added together. This is probably due to the increased transport of fatty acids into the mitochondrion by carnitine (13). Increased oxidation of α-ketoglutarate could provide ATP or GTP needed in the activation of fatty acids (14).

A much greater increase in ketogenesis was seen when glucagon was added to fortified homogenates (Table 2). Glucagon is rapidly catabolized by the liver; thus, the smaller ketogenic effect of glucagon in unfortified homogenates may indicate that fatty acid activation or oxidation was rate-limiting in this system. A slightly greater stimulation in ketone body

TABLE I. Effect of Cofactors on Ketogenesis by Rat Liver Homogenates

| Cofactors Added | Total Ketones (μmoles/gm/90 min) | % of Control |
|---|---|---|
| None | 1.19 ± .08 | 100 |
| Carnitine | 1.56 ± .09 | 131 |
| NAD+ | 1.45 ± .07 | 122 |
| ATP | 1.69 ± .17 | 142 |
| Cytochrome c | 1.37 ± .04 | 116 |
| α-ketoglutarate | 1.62 ± .17 | 137 |
| Carnitine + NAD+ | 1.50 ± .12 | 124 |
| Carnitine + ATP | 1.48 ± .12 | 124 |
| Carnitine + Cytochrome c | 1.76 ± .26 | 148 |
| Carnitine + α-ketoglutarate | 2.70 ± .21 | 227 |
| Carnitine + NAD+ + ATP | 1.89 ± .19 | 159 |
| Carnitine + NAD+ + α-ketoglutarate | 2.36 ± .12 | 198 |
| Carnitine + NAD+ + ATP + Cytochrome c | 1.90 ± .22 | 160 |
| Carnitine + NAD+ + ATP + α-ketoglutarate | 2.58 ± .13 | 218 |
| Carnitine + NAD+ + ATP + Cytochrome c + α-ketoglutarate | 2.40 ± .08 | 202 |

Conditions of the incubations were the same as those described in materials and methods. The values are the mean ± SE of the mean of separate homogenates prepared from three animals. Final concentration of cofactors: dl-carnitine - 1.5 mM; NAD+ - 1 mM; ATP - 1 mM; α-KGA - 10 mM; cytochrome c 15 μM.

TABLE 2. Effect of Glucagon, Cyclic AMP and Theophylline on Rat Liver Homogenates Fortified with Cofactors.

| | Control (mμmoles/gm/90 min) | Δ Due to Addition (mμmoles/gm/90 min) | | |
|---|---|---|---|---|
| | | Glucagon ($3 \times 10^{-5}$M) | Cyclic AMP ($1 \times 10^{-3}$M) | Theophylline ($1 \times 10^{-3}$M) |
| Acyl CoA | 22.0 ± 2.9 | 12.0 ± 4.1 | 16.2 ± 4.6 | 12.5 ± 2.9 |
| Total Ketones | 2540 ± 78 | 364 ± 49 | 282 ± 96 | 588 ± 155 |

Conditions of the incubation were the same as those described in materials and methods. The values are the mean change from the control ± SE of the mean of paired homogenates from six animals. Final concentration of cofactors were: dl-carnitine - 1.5 mM; NAD+ - 1 mM; ATP - 1 mM; α-ketoglutarate - 10 mM; cytochrome c - 15 μM.

production was observed with cyclic AMP and theophylline in fortified homogenates. Long-chain acyl CoA esters were also found to be elevated by these three agents (Table 2) indicating that more fatty acids were made available for activation to their CoA esters.

## Discussion:

The ketogenic effect of glucagon is thought to be mediated by cyclic AMP and may be secondary to the stimulation of a hepatic lipase. Chmelarova et al. (15) have recently reported that hepatic lipase is sensitive to glucagon in vitro. The results reported here demonstrate that glucagon, cyclic AMP and theophylline elevate the levels of acyl CoA esters and stimulate ketogenesis in homogenates. These results tend to support the lipase mechanism for the stimulation of ketogenesis by glucagon. Allen et al. (16) have recently demonstrated that an adenyl cyclase system, which can be stimulated by glucagon and epinephrine, exists in rat liver mitochondria. Stein and Shapiro (17) have found that 15 min. after the intravenous injection of triglycerides to rats, the glycerides were concentrated in liver mitochondria; these triglyceride levels decreased with time indicating that hydrolysis of the lipid was occurring. The presence of a triglyceride lipase in mitochondria has been demonstrated (18, 19). It is suggested that the stimulation of ketogenesis by glucagon in homogenates may be due in part to the activation of mitochondrial adenyl cyclase; since cyclic AMP has previously been shown to stimulate fatty acid oxidation (20), it is tempting to speculate that the evolved cyclic AMP may activate a mitochondrial lipase and/or steps in the activation or oxidation of fatty acids.

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