Inhibition of Glucose Oxidation and Fatty Acid Synthesis in Liver Slices from Fed, Fasted and Fasted–Refed Rats by Glucagon, Epinephrine and Cyclic Adenosine-3′,5′-monophosphate (37325)

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Haugaard and Stadie (1) reported that glucagon or epinephrine decreased acetate incorporation into fatty acids in rat liver preparations. Since many of the functions of these two hormones are mediated through cyclic adenosine-3′,5′-monophosphate (cAMP) it would appear that cAMP is involved in regulation of fatty acid synthesis. Indeed, Berthet (2) reported that cAMP inhibited acetate incorporation into fatty acids and cholesterol in rat liver slices. Recently, Allred and Roehrig (3) showed that cAMP inhibited acetate-1-^{14}C incorporation into total lipids of avian liver slices, and Bricker and Levey (4) reported an inhibition by cAMP of acetate-2-^{14}C incorporation into hepatic fatty acid and cholesterol in liver slices from fed rats.

Hepatic cAMP level appears to be under hormonal control since its concentration is increased by glucagon and decreased by insulin (5, 6). In fasting, secretion of pancreatic glucagon increases, whereas that of insulin declines (7). In contrast, the opposite has been observed during hyperglycemia, following carbohydrate ingestion (8). Consequently, hepatic levels of cAMP appear to be affected by the nutritional state of the animal. Since the role of glucagon, epinephrine or cAMP in regulation of fatty acid synthesis during fasting and/or hyperalimentation has not been defined, we have investigated *in vitro* effects of the two hormones and cAMP on glucose oxidation and fatty acid synthesis by liver slices prepared from fed, fasted, and fasted–refed rats.

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Materials and Methods. Male Holtzman rats ranging in weight from 160 to 180 g were individually housed at 25° and fed a synthetic diet for 7 days before experimentation. The diet contained by weight the following components: 15% crude casein, 78% sucrose, 3% corn oil, 4% USP salt mixture XIV, and a complete vitamin supplement. Food as well as water were available *ad libitum*. After the 7-day dietary adjustment period, the rats were randomly segregated into three treatment groups. The first group continued on the *ad libitum* feeding schedule for an additional 4 days, the second group was fasted for 2 days and the third group was fasted for 2 days and refed for 2 days. At the end of the fasting or feeding periods, five animals from each group were decapitated and liver slices were incubated for 2 hr at 37°, under 95% O_2–5% CO_2, in 25-ml Erlenmeyer flasks containing 3.0 ml of Krebs–Ringer bicarbonate buffer, 30 μM glucose, 0.5 μCi glucose-U-^{14}C and 0.1 unit porcine insulin. Pharmacological levels of the hormones added to this system are shown in Table I. At the end of the incubation period, the substrate incorporation into fatty acid and $^{14}CO_2$ was measured by methods reported previously (9, 10). Since epinephrine, glucagon or cAMP has been shown to stimulate glucose release from the liver (7), the present data were expressed with respect to the final glucose concentration in the incubation media, so that an error due to changes in glucose pool size would be minimized. The glucose concentration in the incubation media was measured using the glucose oxidase method (11). The significance of difference between means was calculated by the Student's t test.

TABLE I. Effect of Glucagon, Cyclic Adenosine-3′,5′-monophosphate and Epinephrine on Fatty Acid Synthesis from Glucose-U-^{14}C in Rat Liver Slices.[a]

Addition to system (M)	Treatment group and metabolite					
	Fed		Fasted		Fasted-refed	
	CO_2	Fatty acids	CO_2	Fatty acids	CO_2	Fatty acids
None	6.66 ± 0.60	1.66 ± 0.24	2.72 ± 0.20[c]	0.027 ± 0.01[c]	8.23 ± 0.40	3.84 ± 0.31[c]
Glucagon						
1.5×10^{-9}	4.64 ± 0.23[b]	0.51 ± 0.17[b]	2.74 ± 0.09[c]	0.025 ± 0.01[c]	5.63 ± 0.28[b]	2.31 ± 0.15[b,c]
1.5×10^{-7}	3.50 ± 0.25[b]	0.31 ± 0.08[b]	2.49 ± 0.25	0.024 ± 0.02[c]	3.98 ± 0.30[b]	1.06 ± 0.12[b,c]
1.5×10^{-6}	2.09 ± 0.27[b]	0.36 ± 0.20[b]	1.86 ± 0.21[b]	0.024 ± 0.01[c]	2.47 ± 0.26[b]	0.56 ± 0.10[b]
3×10^{-4}	1.32 ± 0.06[b]	0.043 ± 0.01[b]	1.26 ± 0.18[b]	0.023 ± 0.02	1.40 ± 0.04[b]	0.044 ± 0.01[b]
cAMP						
1×10^{-3}	1.12 ± 0.07[b]	0.029 ± 0.01[b]	1.21 ± 0.12[b]	0.025 ± 0.02	1.38 ± 0.12[b]	0.038 ± 0.02[b]
Epinephrine						
1×10^{-4}	4.11 ± 0.65[b]	0.59 ± 0.29[b]	2.16 ± 0.03[b]	0.027 ± 0.01[c]	4.82 ± 0.43[b]	1.56 ± 0.22[b,c]

[a] Values are mean ± SEM μmoles of glucose incorporated per gram wet weight tissue per 2 hr.
[b] Indicates significant difference $p < 0.05$, same treatment group.
[c] Indicates significant difference $p < 0.05$, from fed controls, same metabolite.

Results and Discussion. As shown in Table I, glucagon and cAMP markedly reduced glucose oxidation and fatty acid synthesis in liver slices from fed rats. In contrast, fatty acid synthesis from fasted rats was not affected by either hormone. However, cAMP and the two high levels of glucagon reduced glucose oxidation in this treatment group. Furthermore, either compound reduced glucose oxidation and fatty acid synthesis in refed animals. The high level of glucagon and cAMP reduced lipogenesis both in fed and in refed animals to the levels similar to those found in fasted animals. Epinephrine also reduced lipogenesis in fed and in refed animals but its effect was less effective than that of glucagon or of cAMP.

It appears from these data that hepatic glucose oxidation and fatty acid synthesis are regulated by glucagon levels perfusing the liver. It seems probable that these effects are mediated by modification of hepatic cAMP levels, and it seems equally probable that the endogenous levels of hepatic cAMP are markedly increased in fasting animals and as a consequence no further reduction in lipogenesis is observed when glucagon, epinephrine or cAMP were added to the incubation media. In contrast, circulating insulin levels in animals fed or refed the high sucrose diet would appear to reduce hepatic cAMP levels, with a resulting stimulation of lipogenesis. Accordingly, we observed a reversal of this stimulatory effect upon the addition of glucagon or cAMP to the incubation media containing insulin.

The mechanism of cAMP inhibition of hepatic fatty acid synthesis has yet to be determined. However, this effect could be the result of inhibition of enzyme synthesis or of an allosteric effect on enzyme activity.

Summary. Glucagon and cyclic adenosine-3′,5′-monophosphate (cAMP) markedly decreased glucose-U-^{14}C oxidation and conversion into fatty acids in liver slices from fed and fasted–refed rats. Epinephrine was much less effective. Lipogenesis from fasted rats was not reduced by any of these compounds below the level already produced by 2-day fast. The data suggest that hepatic glucagon levels are important in regulating glucose oxidation and its conversion into fatty acids. This effect appears to be mediated via modification of hepatic tissue cAMP levels.

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