STIMULATION OF GLYCOPROTEIN SYNTHESIS BY PHOSPHORYLCHOLINE IN VIVO AND IN LIVER SLICES[1]

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Summary

A stimulation of plasma glycoprotein synthesis and a reduction of liver triglycerides in 2-days choline deficient rats were observed within 2 hr of phosphorylcholine administration. Similar treatment with choline had no such effects. The effect of phosphorylcholine, in contrast to choline, was found to be more striking in liver slices on the incorporation of glucosamine-1-^{14}C into medium proteins. The effect of phosphorylcholine is dependent on the dose used and the temperature and pH of the medium.

Studies with intact animals and liver perfusion systems have shown that there is an impairment of glucosamine-1-^{14}C incorporation into liver and plasma proteins in early choline deficiency (1, 2). As the impairment of glucosamine-1-^{14}C incorporation into the plasma proteins extends to plasma low-density lipoprotein fraction, it has been suggested that synthesis of a specific glycoprotein might constitute a precursor step for the biosynthesis or release of plasma low-density lipoproteins. There is general agreement that the cause of triglyceride accumulation in the liver in choline deficiency is due to a decreased release of triglycerides in the form of plasma lipoproteins (3, 4, 5,). How choline is involved in this process has remained uncertain. The possibility that choline or some of its derivatives may be directly involved in glycoprotein synthesis (1, 2), was the object of our present study. A dramatic effect of phosphorylcholine, in contrast to choline, on the process of glycoprotein synthesis has been observed.

Materials and Methods

Male Wistar rats (200-300g) were used to obtain choline supplemented

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and deficient rats (3) for both in vivo and in vitro experiments. Liver slices (1.0 - 1.4mm thick) were prepared by a tissue slicer (Harvard Apparatus) specially designed (6) to cut slices of fatty tissue. Methods for measuring the triglycerides and protein and for assaying the radioactivity of trichloroacetic acid insoluble proteins after appropriate washings and organic solvent extractions have been described (1). The most pronounced in vivo effect of a single phosphorylcholine injection was obtained at a dosage of 20mg/100g body wt. (10% solution in 0.15M NaCl). However, at this dosage phosphorylcholine causes hemoconcentration (hematocrit value for this group 60.3 $\pm$ S.E. 2.56 compared to saline and choline groups 51.4 $\pm$ 1.02 and 52.8 $\pm$ 1.08 respectively) and accumulation of fluid in the peritoneal cavity. The excess fluid in the peritoneum is perhaps an exudate from plasma because the specific radioactivity of this fluid was found to be close to that of plasma. Injection of choline at this dosage (1.85 times more than phosphorylcholine on a molar basis) had no such effects. When a lower dose of phosphorylcholine (10mg/100g body weight for 5 days, experiment II, Table I) was injected, there was no hemoconcentration or excess peritoneal fluid. Finally, the complications of phosphorylcholine injection to the whole animal were avoided by using the liver slice system, which proved to be highly responsive to phosphorylcholine.

Results and Discussion

The results in Table 1 show that within 2 hr after phosphorylcholine injection into the 2-days choline deficient rats there was 52% increase of plasma glycoprotein synthesis and significant decrease of liver triglycerides (experiment I). Choline treatment showed no such effects. In experiment II when phosphorylcholine and choline were injected daily into the deficient animals for 5 days, both compounds had almost identical effects on plasma glycoprotein synthesis and liver triglycerides. It is possible that 5-day treatment with choline restores the phosphorylcholine level of the choline deficient liver, which is known to be greatly reduced in choline

Table 1. EFFECT OF PHOSPHORYLCHOLINE AND CHOLINE ON PLASMA GLYCOPROTEIN SYNTHESIS AND LIVER TRIGLYCERIDE LEVEL IN VIVO*.

Experiment	Treatment and No. of animals	Dosage	Plasma protein specific activity. Dpm/mg trichloroacetic acid insoluble protein X 10^{-2}	Liver triglyceride. µE triglyceride fatty acids/liver/100g body wt
I 2-days choline deficient rats	Saline (5)	-	16.8 ± 1.45	790 ± 105
	Phosphorylcholine (6)	20mg/100g b. w. one injection	†† 25.5 ± 2.47	† 534 ± 56
	Choline (5)	20mg/100g b. w. one injection	14.8 ± 0.74	719 ± 91
II 5-days choline deficient rats	Saline (3)	-	8.5 ± 0.79	2296 ± 586
	Phosphorylcholine (5)	10mg/100g b. w. 5 injections	† 12.2 ± 0.66	†† 882 ± 180
	Choline (4)	10mg/100g b. w. 5 injections	† 11.1 ± 0.78	†† 500 ± 108

Statistical significance "P" † <0.05, †† <0.01

* In experiment I, saline, choline (Eastman Kodak) and phosphorylcholine, Ca^{++} (Sigma) were injected intraperitoneally and within 5 min 4µc of glucosamine-1-^{14}C (New England Nuclear) in 0.4 ml saline per rat was injected by the same route. Rats were killed after 2 hr and plasma and liver were obtained for analysis. In experiment II, saline, choline and phosphorylcholine were injected each afternoon for 4 days and in the morning of the 5th day followed by similar isotope injection and interval of killing of the rats as in experiment I.

deficiency (7). Choline feeding to the choline deficient animals also corrects the impairment of plasma glycoprotein synthesis (1). Whereas, on a short-term basis (experiment I and slice experiments), choline could not possibly be efficiently converted to phosphorylcholine.

Figure I shows that after a lag period of 1 hr the slices obtained from 2-days choline deficient rats and incubated in presence of phosphorylcholine released proteins in the medium with much higher glucosamine-1-^{14}C radioactivity compared to their controls. Incorporation of glucosamine-1-^{14}C

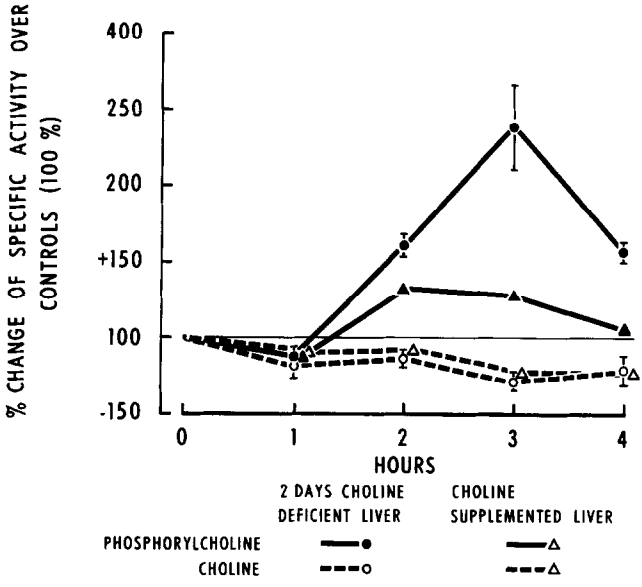

Fig. 1. Phosphorylcholine stimulation of glycoprotein synthesis. Flasks containing 400mg liver slices were incubated with shaking in 2.5 ml of Krebs-Ringer phosphate buffer (with 0.54 mMole/l of Ca^{++}), pH 7.4 at 37°C containing 0.5 µc of glucosamine-1-^{14}C/flask and in the presence of 95% O_2 - 5% CO_2 mixture. At the end of incubation, liver was removed and 1 ml of 20% trichloroacetic acid was immediately added into the medium. Liver slices from the same rat were used for control (saline), phosphorylcholine (1mg or 3.88 µmole) and choline (0.54mg or 3.88 µmole) incubations for different time intervals. Results of 4-6 sets of such experiments have been averaged (with ± S.E.) for the data on choline deficient experiments. The actual specific activities of the medium proteins (dpm/mg protein X 10^{-2}) of the control slices were 1.92 ± 0.42, 2.21 ± 0.30, 4.56 ± 0.92 and 7.42 ± 0.84 respectively for 1 to 4 hr periods and these values have been set for 100%.

into the medium proteins was always less than controls in presence of choline. It is also interesting to note that slices from the choline supplemented animals responded only slightly to phosphorylcholine. This suggests that a decreased level of phosphorylcholine in the choline deficient liver makes the tissue more sensitive for stimulation by phosphorylcholine. An analogous situation is known to exist for the action of vitamin K on the stimulation of plasma prothrombin (a glycoprotein) synthesis. The effect of vitamin K is very pronounced in the vitamin K-deficient rats (8). It has been suggested that the effect of vitamin K is probably exercised at the late- or post-ribosomal level (9, 10). This lends support to the suggestion

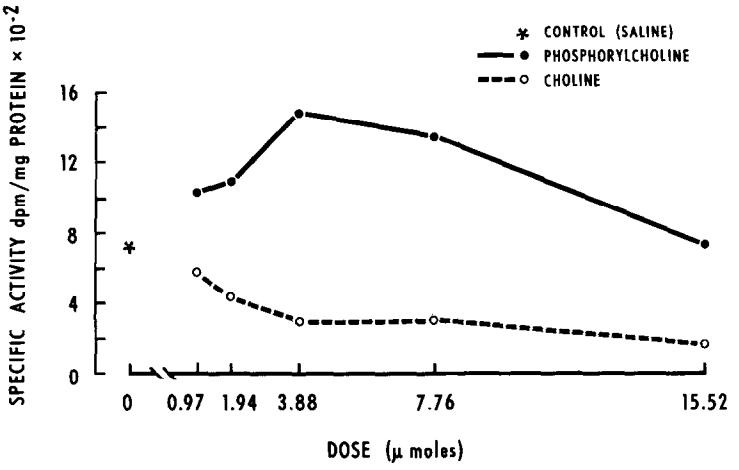

Fig. 2. Dose-response study of phosphorylcholine and choline. Each flask contained 400mg liver slices and 2.5 ml buffer. The incubations in presence of saline (*), phosphorylcholine (-●●-) or choline (--o-o--) were carried out for 3 hr. All other conditions were same as in Fig. 1. All the data shown were obtained from the liver of one 2-days choline deficient rat.

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that a level of regulation exists at the microsomal membranes (1, 2, 11) for the final complexing of the plasma glycoproteins. Figure 2 shows a dose-response of phosphorylcholine and choline on the incorporation of glucosamine-1-^{14}C in the proteins of the medium. Choline at all levels studied was inhibitory compared to the control. The stimulation by phosphorylcholine was maximal between 1.94-3.88 μmole or 0.5-1mg per 400mg liver slices. The phosphorylcholine level in livers of choline supplemented rats is 6.5 μmoles/liver/100g body wt (7), which works out to approximately 0.5-0.7 μmoles/400mg liver tissue. There was over 70% reduction of this level in 2 days of choline deficiency. The present studies indicate that addition of phosphorylcholine to the slices from the choline deficient liver to slightly above (0.97 μmoles) normal physiological range shows a definite stimulatory effect. Table 2 shows the temperature and pH dependence (phosphate buffer is far superior to Tris) of the phosphorylcholine stimulation. The maximum stimulatory effect of phosphorylcholine was observed at 37°C and at pH 7.4.

Table 2. THE TEMPERATURE AND pH DEPENDENCE OF PHOSPHORYLCHOLINE EFFECT ON GLYCOPROTEIN SYNTHESIS*.

	Specific activity (dpm/mg protein X 10^{-2}) of the medium protein		
Temp. (C)	Control	Choline	Phosphorylcholine
10°	4.99	3.71	5.07
37°	14.33	11.16	27.19
45°	8.01	6.15	7.07
pH (Phosphate-buffer)			
5.0	4.05	4.30	2.90
5.8	5.70	4.15	4.15
7.4	14.2	8.20	20.95
8.0	11.65	7.55	14.75
pH (Tris-buffer)			
7.4	7.49	7.93	9.92
8.5	7.57	6.49	9.40
9.0	5.35	3.23	6.89

* Incubation time was 3 hr. Liver slices were from 2-days choline deficient rats. All other conditions were similar to Fig. 1.

The temperature and pH dependence suggests that phosphorylcholine may be involved in some enzymatic reactions responsible for the transfer of amino-sugars into the proteins. This possibility is receiving our present attention. Work is also in progress to identify the medium proteins that are highly labelled with the carbohydrate precursor.

The level and synthesis of phosphorylcholine are greatly reduced in 2 days of choline deficiency (7). But this was not rate limiting for lecithin synthesis and, in fact, there was evidence for increased conversion of phosphorylcholine into lecithin in choline deficiency. This raised the possibility that lecithin formation has distinct priority in choline deficient liver and this occurs in competition with other unknown functions of

phosphorylcholine. The results presented here point out to such alternate function(s) of phosphorylcholine.

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