Vol. 91, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1979 Pages 583-591

Influence of NaF and Na_2PO_3F(MFP) on Glucose Metabolism in Rat Hepatocytes*

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Received October 13, 1979

SUMMARY

Addition of NaF or MFP to rat hepatocytes resulted in a decrease in lactate and in an increase in glucose, 3 and 2-phosphoglycerate production. When dihydroxyacetone was present in the incubation medium both NaF and MFP increased the production of glucose, fructose-1,6-diphosphate, 3 and 2 phosphoglycerate, with a decrease in pyruvate and lactate. In the presence of lactate, glucose production increased only in the presence of MFP, but there was a 8-10 fold increase in the level of phosphoenol pyruvate with both NaF and MFP. The crossover data indicated that the activity of some of the glycolytic enzymes may be inhibited in the presence of NaF and MFP.

Fluoride was one of the first substances known to inhibit glycolysis (1). One of the target enzymes of fluoride inhibition is enolase (E.C. 4.2.1.11) (2). Later other enzymes, eg. phosphoglucomutase (E.C. 2.7.5.1), (3,4), glucose-6-phosphatase (5) (E.C. 3.1.3.9), phosphoglycerol mutase (E.C. 3.1.3.11) (6), and pyruvate kinase (E.C.2.7.1.40) (7) were found to be inhibited by fluoride in vitro. A few studies have been conducted on the effect of fluoride on glucose metabolism in intact mammalian cells. Stossel et al. (8) found that 20 mM NaF increased phosphorylase (E.C. 2.4.1.1) activity and decreased glycogen synthetase (E.C. 2.4.1.11) activity in polymorphonuclear leukocytes without any change in the level of glycogen. Fieg et al. (9) and Millman et al. (10) have shown that NaF interrupts Na^+-K^+ transport in intact red blood cells by inhibiting enolase. Guminska et al. (7) have reported that the addition of NaF to erythrocytes or Ehrlich acites tumor cells could decrease glucose oxidation

* Abbreviations used: MFP, Na_2PO_3F; cAMP, 3'5'cyclic AMP; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenol pyruvate; DHA, dihydroxyacetone.

0006-291X/79/220583-09$01.00/0

via an inhibition of enolase and pyruvate kinase. Shahed et al. (11,12) recently demonstrated that NaF and MFP can stimulate cAMP production, and decrease pyruvate and lactate production in intact rat hepatocytes.

The present study was undertaken to examine the effects of NaF and MFP on glucose metabolism in isolated rat hepatocytes. Hepatocytes were chosen for the following reasons: (1) cAMP production in isolated hepatocytes is stimulated by NaF, MFP, and glucagon (11,12), (b) hepatocytes can be isolated in a viable and hormonally sensitive form, (c) hepatocyte metabolism can be examined in the absence of exogenous factors eg. epinephrine, and (d) the external environment is easily controlled.

Methods

Male Wistar rats (200-240 g) were fed a fluoride deficient diet and distilled water for 5-7 days to reduce the level of liver cAMP prior to the isolation of hepatocytes (11). The isolation and incubation of hepatocytes was as described previously (11).

The total (medium plus cells) concentration of glucose, (13) lactate, (14) and pyruvate (14) was determined using spectrophotometric methods on neutralized perchloric acid extracts of the incubation system.

Liver glycogen was assayed as described by Lee and Whelan (15) on perchloric acid extracts of the incubation system as glucose equivalents (13). The following intracellular metabolites were assayed in neutralized perchloric acid extracts of hepatocytes after separation and concentration of the cells using the separation tubes described by Shahed (16): glucose-6-phosphate and fructose-6-phosphate (17), glucose-1-phosphate (18), and fructose-1,6-diphosphate, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate (19), and 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate (20). Purified MFP was a gift of the Ozark-Mahoning Company. The purified MFP contained 10.35% total fluoride and 0.05% free fluoride.

Results

The data presented in Table I show that in the absence of any carbon source, glucose production increased significantly in the presence of NaF, MFP and glucagon. A corresponding decrease in the level of liver glycogen was also observed (data not shown). Both MFP and glucagon significantly increased glucose production in the presence of lactate or DHA. However, NaF had no effect on glucose production in the presence of lactate but glucose production was increased in the presence of DHA. In the presence of lactate and DHA no

TABLE 1

Effect of NaF, MFP and Glucagon on Glucose Production in Rat Hepatocytes

Substrate	No Addition	NaF (10 mM)	MFP (20 mM)	Glucagon (100 nM)
		μmoles glucose/g wet wt/60 min		
None	32 ± 2	45 ± 3*	65 ± 6*	57 ± 1*
Lactate	186 ± 20	163 ± 42	228 ± 24*	244 ± 5*
DHA	185 ± 8	210 ± 19*	207 ± 10*	249 ± 30*

The results are expressed as means ± S.E.M. of 5 different hepatocyte preparations. The hepatocytes were incubated for 60 min in the presence of 2.5% BSA, 1 mM theophylline and a given substrate (10 mM).

* $p < 0.05$

significant decrease in glycogen was observed (data not shown). It appears that the source of glucose may be affected by the presence or absence of a carbon source. To gain an insight into the possible sites of action of NaF and MFP on glucose metabolism, the concentration of most of the glycolytic intermediates were measured. It can be seen in Table 2 and Figure 1a that in the presence of NaF, and MFP, there was a significant increase in the concentration of G6P, F6P, 3PG, 2PG, PEP, and a decrease in lactate. From the cross-over data in Figure 1a, a decrease in FDP relative to F6P can be seen. Also the data suggest a cross-over between PEP and pyruvate with a relative increase in 3PG and 2PG. These data suggest that one or more of the following enzymes may be partially inhibited by NaF and MFP: phosphoglycerol mutase, enolase, pyruvate kinase, or phosphofructokinase. In the presence of glucagon there is a cross-over between F6P and FDP as well as between PEP and pyruvate. Glucagon is known to induce the phosphorylation and inactivation of phosphofructo kinase (21) and pyruvate kinase (22,23).

The data shown in Figure 1b were obtained in which lactate (10 mM) was added to the incubation system. The data in Figure 1b illustrate that in contrast to

TABLE 2

Effect of NaF and MFP on the Level of

Glycolytic Metabolites in Rat Hepatocytes*

Metabolite	None	NaF (10 mM)	MFP (20 mM)	Glucagon (100 nM)
Glucose-6-phosphate	36 ± 4	50 ± 6**	100 ± 10*	78 ± 30**
Fructose-6-phosphate	15 ± 2	40 ± 6**	46 ± 10**	28 ± 10**
Fructose-1,6-diphosphate	50 ± 6	48 ± 6	93 ± 60**	47 ± 8
3-phosphoglycerate	120 ± 20	625 ± 110**	725 ± 130**	187 ± 20**
2-phosphoglycerate	20 ± 2	63 ± 12**	94 ± 10**	30 ± 4**
Phosphoenol pyruvate	23 ± 2	50 ± 7**	78 ± 20**	40 ± 10**
Pyruvate	2.0 ± 0.2	1.5 ± 1	2.0 ± 0.3	1.0 ± 0.1**
Lactate	9.0 ± 1.4	4.0 ± 0.5**	5.0 ± 1**	2 ± 0.5**

* Rat hepatocytes (5 preparations) were incubated for 60 min in the presence of 1 mM theophylline. The concentrations for all the metabolites except glycogen, glucose, pyruvate, and lactate are expressed as nmoles per gm wet weight. The values for glycogen, glucose lactate, and pyruvate are expressed as μmoles per gm wet weight. The data are expressed as a mean ± S.E.M.

** $p < 0.05$

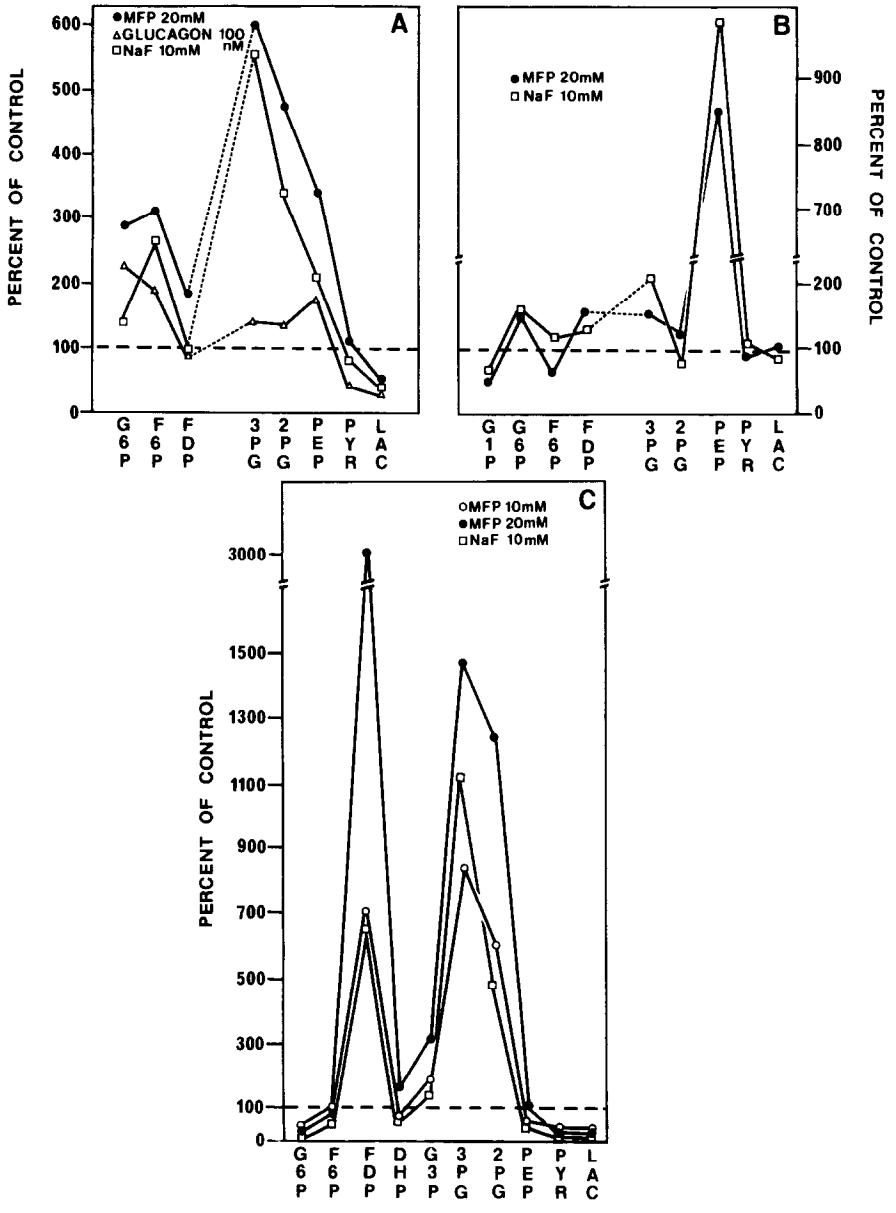

Figure 1, Hepatocytes were incubated in Krebs-Hanseleit buffer pH 7.4 containing 2.5% BSA, and 1 mM theophylline for 60 min at 37°C in a shaking water bath. The data is presented as % change from the control: (a) No carbon source was added, (b) Lactate (10 mM) was incubated in the incubation medium, and (c) DHA (10 mM) was used as a carbon source.

Figure 1a both NaF and MFP caused a 7-10 fold increase in PEP without any significant change in the concentration of either 2-phosphoglycerate or 3-phosphoglycerate. These data are consistent with an inhibition of enolase. A

cross-over between glucose-6-phosphate and glucose-1-phosphate was also seen, suggesting a possible inhibition of phosphoglucomutase. To obtain more information about the action of NaF and MFP on the steps involved prior to enolase in the glycolytic pathway, hepatocytes were incubated with DHA. DHA enters the glycolytic pathway after being phosphorylated. The data in Figure 1c indicate a reduction in the level of G6P, PEP, pyruvate, and lactate in the presence of NaF and MFP. Also a large increase in the level of FDP, 3PG, and 2PG was observed. The data in Figure 1c, suggest that NaF and MFP increase the flux of DHA carbons towards glucose as indicated by an increase in FDP and glucose production. The increase in glucose production may result from an inhibition of phosphoglycerolmutase and enolase.

Discussion

The data presented in Table 1 show that NaF, MFP and glucagon increased glycogenolysis and inhibited glycolysis in hepatocytes from fed rats, in the absence of any added carbon source. Shahed *et al.* (11,12) have reported that both NaF and MFP can increase cAMP accumulation in hepatocytes. Shahed *et al.* (24) have recently found that the activity of liver glycogen phosphorylase *a* is stimulated by NaF and MFP. Stossel *et al.* (8) also observed a stimulation of phosphorylase *a* activity by NaF in polymorphonuclear leucocytes. McGown and Suttie (25) did not observe any increase in the activity of liver phosphorylase *a* when rats were injected with 6 mg NaF/Kg body weight. From the data presented here, it is proposed that NaF and MFP may induce glucose production in rat hepatocytes by increasing the cAMP level and thereby activating phsophorylase *a* and thus increase glycogenolysis.

A dramatic increase in the concentration of 3PG and 2PG followed by relatively minor changes in PEP, pyruvate and lactate suggest possible inhibition of one or more of the following 3 enzymes (a) phosphoglycerate mutase (b) enolase and (c) pyruvate kinase (Table 2, Fig. 1a). A lack of a crossover at each of these steps may be explained on the basis of the unique position of these

enzymes in the glycolytic pathway e.g. these enzymes catalyze consecutive steps. Therefore, an inhibition of both enolase and pyruvate kinase could prevent a crossover at enolase since a build up of PEP would result from an inhibition of the latter enzyme. An inhibition of enolase, pyruvate kinase and phosphoglycerate mutase by NaF in vitro has been previously reported (2,6,7).

A significant increase in glucose production was observed in the presence of MFP and glucagon when lactate was used as a carbon source (Table I). However NaF had no effect on glucose production under these conditions. The data in Figure 1b show a 775% and 900% increase in the concentration of PEP in the presence of NaF and MFP, respectively, followed by an insignificant change in the level of 2PG and 3PG. In contrast to this data, the results in Figure 1a show an increase in the concentration of 3PG and 2PG. These data (Fig. 1b) are therefore consistent with an inhibition of enolase and a stimulation of gluconeogenesis. If the flux of carbon was through glycolysis, then an increase in 3PG and 2PG would be expected (Figure 1a). The increase in PEP can only be explained if the gluconeogenic pathway was turned on. On the basis of the present data (Table 1, Fig. 1b) it is speculated that, part of the glucose may be produced via a gluconeogenic pathway. A lack of an increase in glucose production in the presence of NaF may be caused by inhibition of enolase and other enzymes (see below).

DHA which enters the glycolytic and gluconeogenic pathway at the triose phosphate level can be converted into glucose in the presence of cAMP and glucagon in hepatocytes from both fed and fasted rats (26,27). It has been proposed that glucagon directs DHA towards glucose synthesis by reducing the flux at pyruvate kinase. The results indicate that NaF and MFP stimulate glucose production and reduce lactate and pyruvate production in the presence of DHA (Fig. 1c). When the concentration of all the glycolytic intermediates was measured the following observations were made: (a) an increase in 3PG and 2PG in the presence of NaF and MFP; (b) an increase in FDP in the presence of NaF and MFP, and (c) no change in the concentration of F6P. These results strongly

suggest that (a) utilization of DHA is decreased due to the inhibition of enolase by NaF and MFP; (b) at least part of the glucose produced (Table 1) is from DHA as indicated by a large increase in FDP (showing the flux towards glucose synthesis); and (c) the activity of fructose-1,6-diphosphatase appears to be inhibited. Fructose-1,6-diphosphatase is a regulatory step in gluconeogenesis and its inhibition would reduce the flux of carbons from any substrate such as DHA towards glucose synthesis (26-27). This information is consistent with the proposal that NaF and MFP, by reducing the utilization of DHA through glycolysis at enolase, may direct its flux toward glucose synthesis but the latter may be blocked due to the partial inhibition of fructose-1,6-diphosphatase. The data in Figure 1c do not suggest an inhibition at pyruvate kinase under these conditions, this may be due to a 7-10 fold increase in the concentration of FDP (Fig. 1c). FDP is a positive allosteric activator of pyruvate kinase and reduces the inhibitory effect of cAMP on this enzyme (22).

ACKNOWLEDGEMENT

This study was supported in part by the Grace M. Showalter Trust and a NIDR grant 04387.

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