Effects of Nifedipine and Nicardipine on Glucagon-Stimulated Gluconeogenesis in Primary Cultures of Rat Hepatocytes

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ABSTRACT—The effects of nifedipine and nicardipine on glucagon-stimulated gluconeogenesis from lactate were examined in primary cultures of rat hepatocytes. Nifedipine and nicardipine (10^{-7} – 10^{-5} M) significantly potentiated the glucagon-stimulated gluconeogenesis from lactate by increasing intracellular cAMP levels. In contrast, diltiazem and verapamil did not potentiate the glucagon-stimulated gluconeogenesis. 1-Methyl-3-isobutylxanthine and papaverine also potentiated the glucagon-stimulated gluconeogenesis from lactate. On the basis of these results, possible mechanisms by which nifedipine and nicardipine potentiate the glucagon-stimulated gluconeogenesis will be discussed.

Keywords: Nifedipine, Gluconeogenesis, Primary cultures of rat hepatocytes

Calcium channel blockers such as nifedipine, nicardipine and a number of related agents have been widely used in the management of hypertension and related cardiovascular disease. Although mechanisms of action of these agents on the cardiovascular system have been extensively studied in vitro and in vivo, there have only been a few reports about the effects of these calcium channel blockers on the regulation of glucose metabolism.

Previous studies have shown that dihydropyridine calcium channel blockers, nifedipine and nicardipine, produce mild hyperglycemia in 20 hr-starved streptozotocin-diabetic rats in vivo (1). Since plasma glucose levels are dependent on both hepatic glucose production (glycogenolysis and gluconeogenesis) and peripheral glucose utilization, the mechanism of action of these agents may be related to the stimulation of hepatic glucose production and/or the peripheral glucose utilization (2).

However, in vivo experimental systems have some limitations, for instance that the results are affected by the indirect effects of many endogenous factors (1). Compared to the whole animal, an in vitro system has the advantage that endogenous hormones and other effectors do not influence the process being studied and various hormonal conditions can be tested in one single cell preparation. In addition, high levels of glucagon and catecholamines that may occur in diabetes in vivo increase glucose production by the liver. In the present study, the author examined whether these calcium channel blockers affect the glucagon-stimulated gluconeogenesis in primary cultures of rat hepatocytes.

Liver parenchymal cells were isolated from male Wistar rats weighing 200–250 g by a modification of the procedure of Berry and Friend, which is described in detail elsewhere (3). In brief, the liver was first perfused via the portal vein with a calcium-free Hanks’ 10 mM Hepes buffer (pH 7.4) at 37°C and at a flow rate of 30 ml/min for 10 min. The second step was performed with the same buffer containing 0.025% collagenase and 0.075% CaCl2 at the same flow rate. At the end of the perfusion, the cells were dispersed in calcium-free Hanks’ solution. They were washed three times by slow centrifugation (50 g) for 1 min to remove cell debris, damaged cells and non-parenchymal cells. Routinely, more than 85% of the cells were intact as assessed by the trypan blue exclusion test. Inocula of 1 X 10^6 cells were seeded to 35-mm diameter collagen-coated plastic dishes and incubated with 2 ml of Williams E medium containing 10^{-8} M dexamethasone, 5% calf serum, 100 mg/ml streptomycin, and 100 μU/ml penicillin at 37°C under an atmosphere of 5% CO2 in air. After a 4-hr attachment period, hepatocytes were washed twice with the culture medium and incubated for a further 20 hr.

After 24 hr of culture, hepatocytes were washed
twice with Dulbecco's phosphate-buffered saline (pH 7.4). They were incubated in 1.0 ml of the Hanks-10 mM Hepes buffer (pH 7.2) containing 10 mM lactate as a gluconeogenic substrate for 1 hr at 37°C under an atmosphere of 5% CO₂ in air. An 10-μl aliquot of glucagon and/or calcium channel blockers and phosphodiesterase inhibitors was added simultaneously to the incubation medium. Glucose in the medium (0.5 ml) was determined by the glucose oxidase-peroxidase method. Glucose synthesis from 10 mM lactate has been corrected for synthesis from endogenous metabolites by subtracting the glucose production in the absence of 10 mM lactate from those values in the presence of the substrate (4).

After 1 hr of incubation, gluconeogenesis was terminated by adding 0.1 ml of 1 N HCl. The cells were scraped off with a rubber policeman. Then the cells and medium were transferred to test tubes and heated at 100°C for 3 min to obtain a deproteinized extract. The suspension was centrifuged at 1500 × g for 5 min. The adenosine 3',5'-monophosphate (cAMP) content of the supernatant was determined by the radioimmunoassay procedure of Honma et al. (5). Cellular protein was determined by the method of Lowry et al. using bovine serum albumin as a standard (6).

Bovine serum albumin (fraction v) and collagenase (type II) were obtained from Worthington Biochemical Co. (Freehold, NJ). Glucagon, nifedipine, nicardipine and 1-methyl-3-isobutylxanthine (IBMX) were obtained from Sigma Chemical Co. (St. Louis, MO). The assay kit for cAMP was obtained from Yamasa Co. (Chiba, Japan). All other chemicals were analytical grade.

Statistical significance of differences was determined by the unpaired Student’s t-test. P values less than 0.05 were regarded as statistically significant.

As shown in Fig. 1 (A, B), glucagon produced dose-dependent increases in hepatic gluconeogenesis from 10 mM lactate up to about 160%. The glucagon stimulation of gluconeogenesis from 10 mM lactate was potentiated by 10⁻⁷ - 10⁻⁵ M nifedipine and nicardipine. Each of these dihydropyridine calcium channel blockers by itself did not significantly affect the basal gluconeogenesis from 10 mM lactate (not shown). In contrast, verapamil and diltiazem (10⁻⁷ - 10⁻⁶ M) were not effective in potentiating glucagon-stimulated gluconeogenesis from 10 mM lactate. Omission of Ca²⁺ from the incubation medium did not affect the glucagon-stimulated gluconeogenesis (Fig. 1, A).

Glucagon led to dose-dependent increases in the levels of cAMP when hepatocyte monolayers were incubated in the presence of 10 mM lactate (Fig. 1, C and D). Nifedipine and nicardipine (10⁻⁷ - 10⁻⁶ M) potentiated the glucagon stimulation of cAMP levels in

![Fig. 1. The effects of nifedipine and nicardipine on glucagon-stimulated gluconeogenesis from 10 mM lactate and glucagon-stimulated cAMP levels in primary cultured hepatocytes. Primary cultured hepatocytes were incubated with the substrate and agents (10 μl) for 1 hr in Hanks-10 mM Hepes buffer (pH 7.2). After the incubation, the supernatants (0.5 ml) were assayed for glucose. Results are expressed as nanomoles glucose formed/hr/mg of cellular protein (A, B). The basal value is 322 ± 15 nmol/hr/mg protein. The cAMP levels in the cultured hepatocytes were determined by radioimmunoassay as described in the text. They were expressed as picomoles of cAMP/mg of cellular protein (C, D). The basal value is 2.50 ± 0.03 pmol/mg protein. Results are the mean ± S.E.M. of 3–4 cell preparations. Values significantly different from the respective control values (glucagon alone) are indicated as * (P < 0.05). (A): Glucagon (Gln), Gln without extracellular Ca²⁺ ( ), Gln + 10⁻⁷ M Nifedipine (Nif, ), Gln + 10⁻⁷ M Nicardipine (Nic, ); (B): Gln ( ), Gln + 10⁻⁷ M Nicardipine ( , ); Gln + 10⁻⁶ M Nic ( ); (C): Gln ( ), Gln + 10⁻⁷ M Nif ( , ); Gln + 10⁻⁶ M Nif ( , ); (D): Gln ( ), Gln + 10⁻⁷ M Nic ( , ), Gln + 10⁻⁶ M Nic ( , ), Gln + 10⁻⁵ M Nic ( , ).]
the hepatocyte monolayers. Treatment with nifedipine or nicardipine alone did not significantly affect the basal cAMP levels. Verapamil and diltiazem \((10^{-7} - 10^{-6} \text{ M})\) did not potentiate the glucagon-stimulated cAMP levels in the primary cultures of hepatocytes (not shown).

Treatment of primary cultured hepatocytes with cAMP phosphodiesterase inhibitors, IBMX and papaverine, also potentiated the glucagon stimulation of gluconeogenesis from lactate in a dose-dependent manner (Fig. 2). These cAMP phosphodiesterase inhibitors alone did not significantly influence basal gluconeogenesis from 10 mM lactate (not shown).

It is well-known that the liver is the major site of gluconeogenesis. Glucagon as well as catecholamines contribute to the enhanced synthesis of glucose in adult rat liver. There is general agreement that the stimulation of gluconeogenesis by glucagon and catecholamines (\(\beta\)-action) is mediated through changes in the intracellular concentration of cAMP (7–10).

As shown in Fig. 1, nifedipine and nicardipine potentiated the glucagon-stimulated gluconeogenesis from lactate in primary cultured rat hepatocytes. In contrast, non-dihydropyridine calcium channel blockers, verapamil and diltiazem \((10^{-7} - 10^{-6} \text{ M})\), did not affect the glucagon-stimulated gluconeogenesis. Much higher concentrations of these calcium channel blockers \((10^{-5} - 10^{-3} \text{ M})\) did not potentiate but actually inhibited the glucagon-stimulated gluconeogenesis (not shown). Furthermore, the glucagon stimulation of gluconeogenesis was not affected by the absence of extracellular calcium ions (Fig. 1). These results suggest that reduction of Ca\(^{2+}\) entry into hepatocytes does not account for the effect of dihydropyridine calcium channel blockers on the glucagon-stimulated gluconeogenesis. Different mechanism(s) may be involved in the response.

Figure 1 also showed that there was an elevation of cAMP levels which was paralleled by an increase in glucose synthesis from lactate in hepatocyte monolayers. In addition, it has been reported that dihydropyridine calcium channel blockers inhibit both calmodulin-sensitive and calmodulin-insensitive forms of cAMP phosphodiesterase activity from various tissues (e.g., hepatocytes, brain, intestine, etc.) with IC\(_{50}\)s in the micromolar range. However, non-dihydropyridine calcium channel blockers could not bring about any modification in these enzyme activities (11, 12). From the results in Fig. 1 and the literature, a possible explanation for the mechanism by which nifedipine and nicardipine potentiate the glucagon-stimulated gluconeogenesis is related to the increase in the tissue levels of cAMP through the inhibition of cAMP phosphodiesterase. Accordingly, treatment of the hepatocytes with phosphodiesterase inhibitors, IBMX and papaverine, also produced marked potentiation of glucagon-stimulated gluconeogenesis from lactate (Fig. 2).

Furthermore, the author observed that the gluconeogenic effect of the \(\beta\)-adrenergic agonist isoproterenol was also potentiated by nifedipine and nicardipine in primary cultures of hepatocytes. This potentiation appeared to be dependent on the increase in the tissue levels of cAMP (data should be published). Therefore, the action of these calcium channel blockers on the glucagon- and the isoproterenol-induced gluconeogenesis seems to be mediated by the changes of cAMP levels.

These pharmacological studies support the hypothesis that nifedipine and nicardipine produced mild hyperglycemia, at least in part, by potentiating glucagon- and catecholamine-stimulated gluconeogenesis in streptozotocin-induced diabetic rats in vivo.

**Fig. 2.** The effects of IBMX and papaverine on glucagon-stimulated gluconeogenesis from 10 mM lactate in primary cultured hepatocytes. Experimental details are the same as described in Fig. 1. Results are expressed as nmol glucose formed/hr/mg of cellular protein. Results are the means ± S.E.M. of 3–4 cell preparations. Values significantly different from the respective control values (glucagon alone) are indicated as *\((P < 0.05)\). Glucagon (Gln), Gln + 1 × 10^{-3} M IBMX (Δ), Gln + 5 × 10^{-3} M IBMX (▲), Gln + 10^{-5} M papaverine (Pap, □), Gln + 10^{-4} M Pap (■).
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