Effects of Calcium Channel Blockers and Hydralazine on Plasma Glucose Levels in Streptozotocin-Induced Diabetic Rats In Vivo

Katsuyoshi SUNAGA and Masahiko OGIHARA*

Group of Biochemical Pharmacology, Faculty of Pharmaceutical Sciences, Josai University, 1-1, Keyakidai, Sakado, Saitama 350-02, Japan

Accepted December 7, 1989

Abstract—Effects of calcium channel blockers from structurally different classes and hydralazine on plasma glucose levels were examined in streptozotocin-induced diabetic rats in vivo. Non-dihydropyridine calcium channel blockers (verapamil, diltiazem, 1.0–10 mg/kg, i.p.) did not significantly affect the basal plasma glucose level, and dihydropyridine calcium channel blockers (nifedipine, 0.1–0.3 mg/kg, i.p.; nicardipine, 0.35–0.70 mg/kg, i.p.) caused mild hyperglycemia, which was blocked by the administration of the β-adrenoceptor antagonist propranolol. In contrast, hydralazine markedly produced hyperglycemia, which was also inhibited by the combined administration of propranolol. The selective α1-adrenoceptor antagonist prazosin greatly potentiated the hydralazine-induced hyperglycemia. Isoproterenol alone showed hyperglycemia similar to that of hydralazine. Hexamethonium (40 mg/kg, i.p.), a ganglionic blocker, blocked the hydralazine-induced hyperglycemia. There was a negative correlation between the hyperglycemic effect and the blood pressure lowering effect by different doses of hydralazine in streptozotocin-diabetic rats, but not in normal rats. These results suggest that endogenous catecholamines are involved in the hydralazine-induced hyperglycemia through the interaction with β-adrenoceptors in streptozotocin-diabetic rats in vivo.

Calcium channel blockers and hydralazine are widely used to treat essential hypertension. The mechanisms of action of these agents on the cardiovascular system have been extensively studied in vitro and in vivo (1–4). However, only a few studies have been reported with respect to the effects of calcium channel blockers as well as hydralazine on the regulation of blood glucose levels (5–7).

The preceding report showed that calcium channel blockers and hydralazine impaired intravenous glucose tolerance and potentiated epinephrine-induced hyperglycemia in 20 hr-starved normal rats in vivo. These effects of calcium channel blockers and hydralazine have been suggested to be related to the reduction in insulin action (6). During the course of these studies, we found that dihydropyridine calcium channel blockers produced a mild degree of hyperglycemia, and that hydralazine caused marked hyperglycemia in 20 hr-starved streptozotocin-diabetic rats in vivo. Thus, the main purpose of the work described here is to elucidate the mechanism(s) responsible for the hyperglycemic effect of hydralazine in 20 hr-starved streptozotocin-diabetic rats in vivo.

Materials and Methods

Animals: Male Wistar rats weighing about 200 g (6 weeks old) were used. They were housed under controlled conditions of light (12/12-hr light/dark cycle) and temperature (24±2°C). Rats were given free access to food (Oriental Yeast Co., Ltd., Japan) and water before experiments.

Experimental procedures: Diabetes was induced by a single intravenous injection of streptozotocin (45 mg/kg in 0.1 M citrate buffer, pH 4.5) to 24 hr food-deprived rats (6–7 weeks old) under sodium pentobarbital anesthesia (45 mg/kg, i.p.). Non-fasting
plasma glucose levels were determined to judge the severity of diabetes 24 hr after the injection of streptozotocin (8). Then, the rats were starved for 20 hr before the experiments. A polyethylene canula (PE 20) was surgically inserted into the left carotid artery of normal and streptozotocin-diabetic rats under pentobarbital anesthesia. Each canula was flushed with heparinized saline (100 u/ml). The arterial catheter from each rat was attached to a microtransducer (Model CP-02, Century Technology Co., U.S.A.), and the blood pressure were recorded on a polygraph.

Blood samples (0.04 ml) were withdrawn serially from the cut-end of the tail and mixed with 10 mM EDTA-10 mM KF saline. After centrifugation (2500 rpm, 5 min), plasma glucose levels were determined by glucose oxidase using commercially available diagnostic kits (Glucose Test Wako, Osaka, Japan).

For the determination of liver glycogen, 20 hr-fasted streptozotocin-diabetic rats were killed by decapitation after the administration of hydralazine. The liver specimens were rapidly excised from streptozotocin-diabetic rats and frozen under liquid nitrogen. Hepatic glycogen level was measured by the method of Seifter et al. (9).

**Drugs:** Calcium channel blockers, hydralazine and propranolol were obtained from Sigma (St. Louis, MO). Streptozotocin and epinephrine hydrochloride were also purchased from Sigma. Phentolamine (Ciba-Geigy) and prazosin hydrochloride (Sigma) were used as a-adrenoceptor blocking agents. All other reagents were analytical grade. Dihydropyridine calcium channel blockers were protected from light during the experiments.

**Statistical analyses:** Statistical significance of differences was evaluated by an unpaired, two-tailed Student's t-test. The linear regres-

---

**Fig. 1.** Effects of nicardipine (A), nifedipine (B) and hydralazine (C) on plasma glucose levels in 20 hr-starved streptozotocin-diabetic rats in vivo. Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). Blood samples were withdrawn serially from the cut-end of the tail. Dihydropyridine calcium channel blockers and hydralazine were given at the time zero. Each point and bar represents the mean±S.E.M. for the indicated number (n) of observations. Abscissa: hours after nicardipine, nifedipine and hydralazine. Ordinate: plasma glucose levels shown as percentages of the initial values. A: nicardipine (mg/kg, i.p.): 0.35 (△, n=5), 0.70 (□, n=5), propranolol (5 mg/kg, s.c.) + 0.70 (■, n=5); saline (○, n=6); initial plasma glucose level was 170.6±13.7 mg% (n=16). B: nifedipine (mg/kg, i.p.): 0.10 (○, n=6), 0.20 (△, n=6), 0.30 (□, n=5), propranolol (5 mg/kg, s.c.) + 0.20 (■, n=5); initial plasma glucose level was 185.3±10.8 mg% (n=17). C: hydralazine (mg/kg, i.p.): 1.0 (○, n=5), 5.0 (△, n=6), 10 (□, n=5); initial plasma glucose level was 165.8±10.8 mg% (n=16). * and **: significantly different from the saline control at P<0.05 and P<0.01, respectively.
sion coefficients were calculated by using the least-squares method. A $P<0.05$ was considered to indicate a significant difference.

Results

As shown in Fig. 1A and B, in vivo administration of nicardipine and nifedipine produced dose- and time-dependent mild hyperglycemia in 20 hr-starved streptozotocin-diabetic rats. The hyperglycemia was almost completely blocked by simultaneous administration of the $\beta$-adrenoceptor blocker propranolol (5 mg/kg, i.p., typical data). In vivo administration of verapamil (1.0–10 mg/kg, i.p.) and diltiazem (1.0–10 mg/kg, i.p.) did not significantly affect the basal plasma glucose level in 20 hr-starved streptozotocin-diabetic rats (data not shown). In contrast, hydralazine markedly increased the basal plasma glucose level in a dose-dependent manner (Fig. 1C). Significant hyperglycemia was already observed 60 min after the administration of hydralazine (5 mg/kg, i.p.). The hepatic glycogen levels (mg/g liver, $n=5–6$) were as follows: 8.65±0.82 (0 min), 8.52±0.92 (30 min), 11.64±2.27 (60 min), 3.33±1.39 (120 min) and 4.37±0.55 (180 min). Significant decreases in hepatic glycogen contents were observed an 120 and 180 min after the administration of hydralazine (5 mg/kg, i.p., $P<0.05$). The doses of calcium channel blockers and hydralazine were selected on the basis of previous results, which showed glucose intolerance and potentiation of epinephrine-induced hyperglycemia in 20 hr-starved normal rats (6).

To examine possible involvements of $\alpha$- and $\beta$-adrenoceptors, the effects of prazosin (or phentolamine) and/or propranolol on the hydralazine-induced hyperglycemia was
studied. Coadministration of the non-selective α-adrenoceptor blocker phentolamine (2 mg/kg, i.p.) with hydralazine did not significantly alter the hydralazine-induced hyperglycemia (Fig. 2A). However, the hydralazine-induced hyperglycemia was greatly potentiated by the simultaneous treatment of streptozotocin-diabetic rats with the selective α₁-adrenoceptor blocker prazosin (2 and 4 mg/kg, i.p.). Combined administration of the β-adrenoceptor blocker propranolol (2.5 and 5 mg/kg, s.c.) with hydralazine almost completely blocked the hydralazine-induced hyperglycemia. These α- and β-adrenoceptor blockers alone did not significantly affect the basal plasma glucose level in streptozotocin-diabetic rats in vivo (Fig. 2B). Since the hydralazine-induced hyperglycemia was suggested to be mediated through β-adrenergic receptors, we examined the effect of the typical β-adrenoceptor agonist isoproterenol on the plasma glucose level in streptozotocin-diabetic rats in vivo. Isoproterenol (0.1 and 0.2 mg/kg, s.c.) markedly produced hyperglycemia in the streptozotocin-diabetic animals (Fig. 2B). Simultaneous administration of propranolol (5 mg/kg, s.c.) almost completely prevented the effects of isoproterenol (not shown).

To assess the possible involvement of endogenous catecholamine, the effect of hexamethonium, a ganglionic blocker, on the hydralazine-induced hyperglycemia was studied. As shown in Fig. 3, hexamethonium (40 mg/kg, i.p.) significantly blocked the hydralazine-induced hyperglycemia. In contrast, simultaneous administration of hexamethonium (20 and 40 mg/kg, i.p.) did not reduce the isoproterenol-induced hyperglycemia (not shown).

However, interpretation of these results seems to be complicated by a modest fall in blood pressure. Therefore, we examined the relationship between blood pressure response and plasma glucose response by these antihypertensive agents. For this purpose, time-courses of the effect of calcium channel blockers and hydralazine (with α- or β-blocker) on the mean arterial pressure were measured in normal and streptozotocin-diabetic rats. Basal mean arterial blood pressures of normal and streptozotocin-diabetic rats were 90.5±2.5 (n=13) and 90.4±2.5 (n=22) mmHg, respectively. It was found to be unaltered by diabetes. The maximal blood pressure response to these hypotensive agents occurred within a few minutes after i.p. injection (data not shown). Figure 4 shows the correlation between the maximal decrease in mean arterial pressure and maximal increase in plasma glucose by calcium channel blockers and hydralazine in normal and streptozotocin-diabetic rats. There was no correlation between the reduction in blood pressure and hyperglycemic response in normal rats, which were given calcium channel blockers or hydralazine. In contrast, a significant correlation was obtained between the reduction in the mean arterial pressure response and the hyperglycemic response in
Fig. 4. Relationship between maximal decrease in mean arterial blood pressure and maximal increase in plasma glucose levels in 20 hr-starved normal (A) and streptozotocin-diabetic (B) rats, which were given calcium channel blockers, hydralazine and/or sympatholytic agents in vivo. Normal and streptozotocin-diabetic rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). Heparin-filled catheters were placed in the left carotid artery for measurement of mean arterial pressure. After the administration of calcium channel blockers and/or hydralazine with or without α- and β-adrenoceptor blockers, maximal changes in mean arterial pressure were determined and plotted against maximal changes in plasma glucose levels based on the original data in Figs. 1–3. Abscissa: maximal decrease in mean arterial blood pressure (initial %). Ordinate: maximal increase in plasma glucose levels (initial %). A: Normal rats, \( y = -0.247x + 135.8 \) (\( r = -0.421 \), N.S.). B: Streptozotocin-diabetic rats, (a) \( y = -1.95x + 237.9 \) (\( r = -0.780 \), P<0.05), (b) \( y = -0.240x + 136.8 \) (\( r = -0.340 \), N.S.). ○: calcium channel blockers and/or α- and β-blocker. ★: hydralazine and/or α- and β-blocker. Abbreviations used: N.S.: not significant, r: correlation coefficient, D: diltiazem, H: hydralazine, P: propranolol, Ph: phentolamine, Nf: nifedipine, NC: nicardipine, V: verapamil.

Discussion

Plasma glucose levels are dependent on both glucose production (i.e., glycogenolysis and gluconeogenesis) and peripheral glucose utilization (10, 11). The former increases, whereas the latter decreases plasma glucose levels. These metabolic processes are influenced by many factors such as hormones, neurotransmitters, and some drugs. Mechanisms responsible for the hydralazine-induced hyperglycemia in streptozotocin-diabetic rats (Fig. 1) should be explained in terms of the changes in these metabolic activities.

As shown in Fig. 2A, hydralazine-induced hyperglycemia is almost completely blocked by the β-adrenoceptor antagonist propranolol and markedly potentiated by the selective \( \alpha_1 \)-antagonist prazosin. The administration of prazosin with hydralazine may unmask the β-adrenoceptor-mediated action in streptozotocin-diabetic rats. These results suggest that hydralazine produces hyperglycemia through β-adrenergic receptors rather than \( \alpha_1 \)-adrenergic receptors in streptozotocin-diabetic rats. In accordance with the hypothesis, a similar hyperglycemia was observed when the typical β-adrenoceptor agonist isoproterenol was administered to the diabetic animals. It has been reported that peripheral glucose utilization is reduced by β-adrenergic stimulation (10). However, isoproterenol (0.10 mg/
kg, s.c.) dose not significantly affect basal plasma glucose level in 20 hr-starved normal rats. In contrast, isoproterenol produced hyperglycemia (4-fold increase above the basal level) in 20 hr-starved normal rats when the circulating insulin was acutely neutralized by anti-insulin serum (M. Ogihara et al., unpublished observations). In these regards, the reason why isoproterenol did not cause marked hyperglycemia in 20 hr-starved normal rats may be largely due to the β-adrenoceptor-mediated insulin secretion by the β-cells of the pancreatic islets. The insulin may antagonize β-adrenoceptor-mediated inhibition of the peripheral glucose utilization in normal rats.

Catecholamines have both α- and β-action that produce the same final effect on the glycogenolysis in the liver (2). It has been reported that the control of hepatic glycogen breakdown is mainly mediated by α-1-adrenoceptors in normal adult rats (13–15) and by β-adrenoceptors in streptozotocin-diabetic rats (16). As described in the text, the changes in hepatic glycogen may lead to an increase in plasma glucose levels by i.p. hydralazine. For the same reason, β-adrenoceptor-mediated activation of hepatic gluconeogenesis might be associated with the hydralazine-induced hyperglycemia in streptozotocin-diabetic rats. However, the relative contribution of these metabolic changes in hydralazine-induced hyperglycemia is still unknown.

In addition, the hydralazine-induced hyperglycemia is suggested to be mediated by endogenous catecholamines, since hexamethonium, which is known to inhibit the release of endogenous catecholamines from the adrenal medulla (17), significantly blocked the hydralazine-induced hyperglycemia (Fig. 3). The catecholamines may be released to compensate the moderate reduction in blood pressure induced by hydralazine (or probably by dihydropyridine calcium channel blockers). Sympathetic activation and the release of epinephrine from the adrenal medulla in response to hypotensive stress have been reported to be an effective stimulus of hyperglycemia (18). In contrast, hexamethonium (20 and 40 mg/kg, i.p.) did not reduce the hyperglycemic effect of isoproterenol, which directly activates β-adrenoceptors almost exclusively.

As shown in Fig. 4, hydralazine seems to produce hyperglycemia dependent on the reduction in blood pressure. Dihydropyridines and non-dihydropyridines did not seem to cause hyperglycemia dependent on the reduction in blood pressure in streptozotocindiabetic rats, although dihydropyridines have a higher hyperglycemic effect (β-blocker-sensitive, Fig. 1) than non-dihydropyridines. These differences in hyperglycemic response might be related to the fact that calcium channel blockers interfere with stimulus-secretion coupling in glands and nerve endings (19). Clearly, further studies will be necessary to define more precisely the mechanisms by which hydralazine and calcium channel blockers affect plasma glucose levels in the normal and altered metabolic state (i.e., streptozotocin-induced diabetes, glucose load, epinephrine-induced hyperglycemia, etc.).

In conclusion, the hyperglycemic response to hydralazine (and the dihydropyridine calcium channel blockers) may be due to indirect consequences of sympatho-adrenal activation in response to the marked systemic hypotension in the streptozotocin-diabetic rats. During these hyperglycemia, endogenous catecholamines may play an important role in the inhibition of peripheral glucose utilization and/or the activation of hepatic glycogenolysis and gluconeogenesis mainly through β-adrenoceptors.

Acknowledgments: We are grateful to Dr. R. Ishitani for his helpful advice.

References
1 Triggle, D.J. and Janis, R.A.: Calcium channel ligands. Annu. Rev. Pharmacol. Toxicol. 27, 347–369 (1987)
2 Chin, J.H.: Differential sensitivity of calcium channels to dihydropyridines. Biochem. Pharmacol. 35, 4115–4123 (1986)
3 Nakaya, H., Hattori, Y., Nakano, Y. and Kanno, M.: Cardiac versus vascular effects of a new dihydropyridine derivative, CV-4093. In vitro comparison with other calcium antagonists. Eur. J. Pharmacol. 146, 35–43 (1988)
4 Wymson, J.C., Gross, G.J., Brooks, H.L. and Warltier, D.C.: Differential effects of nifedipine, nicorandil and nitroglycerin on the pressor responses elicited by selective alpha-1 and
alpha-2 adrenoceptor agonists in conscious dogs. J. Pharmacol. Exp. Ther. 241, 846–854 (1987)

5 Bhatnagar, S.D., Amin, M.M.A. and Al-Yusuf, A.R.: Diabetogenic effects of nifedipine. Br. Med. J. 289, 19 (1984)

6 Ogihara, M.: Effects of calcium channel blockers and hydralazine on epinephrine-induced hyperglycemia in vivo. Japan. J. Pharmacol. 50, 141–147 (1989)

7 Kanatsuna, T., Nakano, K., Mori, H., Kano, Y., Nishioka, H., Kajiyama, S., Kitagawa, Y., Yoshida, T., Kondo, M., Nakamura, N. and Aochi, O.: Effects of nifedipine on insulin secretion and glucose metabolism in rats and in hypertensive Type 2 (non-insulin dependent) diabetics. Arzneimittelforschung 35, 514–517 (1985)

8 Ogihara, M., Tokumitsu, Y. and Ui, M.: Metabolic alterations in normal and streptozotocin-diabetic rats in vivo: Influence of prolonged starvation. Japan. J. Pharmacol. 34, 307–311 (1984)

9 Seifter, S., Dayton, S., Novic, B. and Muntwyler, E.: The estimation of glycogen with the anthrone reagent. Arch. Biochem. Biophys. 25, 191–200 (1950)

10 Shikama, H. and Ui, M.: Adrenergic receptor and epinephrine-induced hyperglycemia and glucose tolerance. Am. J. Physiol. 229, 962–968 (1975)

11 Shikama, H. and Ui, M.: Metabolic background for glucose tolerance: mechanism for epinephrine-induced impairment. Am. J. Physiol. 229, 955–961 (1975)

12 Exton, J.H. and Harper, S.C.: Role of cAMP in the actions of catecholamines on hepatic carbohydrate metabolism. In Advances in Cyclic Nucleotide Research, Edited by Drummond, G.I. et al., Vol. 5, p. 519–532. Raven Press, New York (1975)

13 Hutson, N.J., Brumley, F.T., Assimacopoulos, F.D., Harper, S.C. and Exton, J.H.: Studies on the α-adrenergic activation of hepatic glucose output. J. Biol. Chem. 251, 5200–5208 (1976)

14 Goodhart, M., Ferry, N., Geynet, P. and Hancun, J.: Hepatic α₁-adrenergic receptors show agonist-specific regulation by guanine nucleotides. Loss of nucleotide effect after adrenalectomy. J. Biol. Chem. 257, 11577–11583 (1982)

15 Nakamura, T., Tomita, A., Noda, C., Shimoji, M. and Ichihara, A.: Acquisition of a beta-adrenergic response by adult rat hepatocytes during primary culture. J. Biol. Chem. 258, 9283–9289 (1983)

16 Bitensky, M.W., Gorman, R.E. and Neufeld, A.H.: Selective effects of insulin on hepatic epinephrine responsive adenyl cyclase activity. Endocrinology 90, 1331–1335 (1972)

17 Yajima, M. and Ui, M.: Hypoglycemia induced by α-adrenergic stimulation during alkalosis. Eur. J. Pharmacol. 41, 93–102 (1977)

18 Himms-Hagen, J.: Sympathetic regulation of metabolism. Pharmacol. Rev. 19, 367–461 (1967)

19 Pinto, J.E.B. and Trifaro, J.M.: The different effects of D-600 (methoxyverapamil) on the release of adrenal catecholamines induced by acetylcholine, high potassium or sodium deprivation. Br. J. Pharmacol. 57, 127–132 (1976)