HYPERGLYCEMIC EFFECT OF HYDRALAZINE IN RATS

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The effect of hydralazine on the alteration of blood sugar, tissue glycogen and blood cAMP levels in rats were investigated. Hydralazine was found to increase blood sugar level in intact rats when administered *i. p.* On the other hand, this hyperglycemia was partially blocked either by the treatment of intact rats with propranolol, β -adrenergic blocker, or by adrenalectomy. Hydralazine treatment increased blood cAMP level, leading to the hyperglycemia, because pretreatment with phentolamine partially blocked the hydralazine-induced hyperglycemia.

Keywords—Hydralazine; hyperglycemia; propranolol; phentolamine; cyclic AMP; glycogen; blood sugar

INTRODUCTION

In 1966, Fortney¹⁾ reported that intravenous injection of hydralazine hydrochloride caused a transient hyperglycemia, followed by a severe hypoglycemia and significant depletion of hepatic glycogen level in dogs. Hydralazine has potent peripheral vasodilative effect and causes a persistent reduction of arterial blood pressure.^{2,3)} This drug is known to inhibit the hypertensive actions of angiotensin, serotonin and posterior pituitary hormones.

In spite of numerous reports on the pharmacological actions of hydralazine, little is known about the effect of hydralazine on carbohydrate metabolism. On the other hand, hyperglycemia has been reported as one feature of toxic actions of Guthion,⁴⁾ paracetamol,⁵⁾ parathion,⁶⁾ amphetamine⁷⁾ and solanine.⁸⁾

The purpose of the present study was to investigate the *in vivo* effect of hydralazine on tissue glycogen, blood sugar and cyclic 3', 5'-adenosine monophosphate (cAMP) levels in relation to hydralazine concentration in plasma of rats.

MATERIALS AND METHODS

Animals and Chemicals—Male and female

Wistar rats weighing 200—230g were housed in temperature (23°) under light-dark cycle (lights on at 8:00 AM and off at 8:00 PM). They were maintained on a commercial diet and water *ad libitum*. Anthrone and glycogen were purchased from E. Merck, Darmstadt, Germany. Anhydrous glucose and *p*-methoxybenzaldehyde (PMBA) from WAKO Pure Chemicals Co., Tokyo. Propranolol from ICI Pharm. KK; phentolamine from Nippon Ciba-Geigy Co., Ltd.; cAMP kit from Yamasa Ltd., Co., Chiba, Japan.

Determination of Blood Sugar and Tissue Glycogen—Blood sugar was determined according to the anthrone method⁹⁾ after deproteinization with Ba(OH)₂ and ZnSO₄. In the studies of glycogen determination, rats were sacrificed by decapitation, and tissues were excised as quickly as possible and frozen immediately with dry ice. Because of the uneven distribution of glycogen in the rat heart, cardiac glycogen was determined on arterial portions. Two hemidiaphragms and duplicate specimens of hepatic and cardiac tissues were analyzed simultaneously according to the method of Kobayashi *et al.*¹⁰⁾

Determination of Blood cAMP Level—cAMP level in rat whole blood was determined using

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TABLE I. *Effect of Hydralazine Treatment on the Tissue Glycogen Levels in Rats*

Tissue	Glycogen (mg/g tissue wet wt.)		<i>p</i> ^{b)}
	Control (7)	Hydralazine (6)	
Liver	35.90 ± 4.25 ^{a)}	6.80 ± 2.89	< 0.001
Diaphragm	1.42 ± 0.45	0.26 ± 0.04	< 0.05
Heart	0.96 ± 0.10	3.89 ± 0.29	< 0.01

a) Mean ± S. E. Figures in parentheses indicate the number of animals used. b) Hydralazine treated vs. control.

RIA kit for clinical purpose with ¹²⁵I succinyl cAMP tyrosine methylester according to the method of Honma *et al.*¹¹⁾

Determination of Plasma Level of Hydralazine in Rats—Plasma level of hydralazine was determined according to the method of Zak *et al.*¹²⁾ with minor modifications as follows. Whole

blood of a rat after hydralazine treatment was withdrawn from the abdominal vein into a heparinized tube and the plasma was separated by gentle centrifugation at 4°. An aliquot of 2 ml of plasma was removed to another test tube and 0.1 ml of 6N HCl was added. The plasma separated was frozen and stored until use.

FIG. 1. *Effect of Hydralazine Treatment on Blood Sugar Level in Male and Female Rats*

Animals were administered hydralazine (20 mg/kg, i.p.) and blood sugar level was determined up to 8 hr after the drug treatment. (●), male; (○), female. Each point represents mean ± S. E. (vertical line). Figures in parentheses indicate the ratio of hyperglycemic rats to total rats used. Statistical significance between male and female rats was shown at each point.

* $p < 0.001$, ** $p < 0.01$.

Two ml of 0.4N HCl, 0.1 ml of 10% ascorbate and 0.2 ml of 1% PMBA were added successively to the plasma and mixed. After incubation at 37° for 30 min, the mixture was adjusted to pH 9.0 with NaOH solution, and extracted with 10 ml of benzene-methanol (3:7, v/v) mixture by shaking for 20 min, followed by centrifugation. The organic phase of the mixture after separation was evaporated to dryness by flushing with nitrogen gas and the residue was dissolved in a mixture consisting of 1 ml of *n*-heptane and 1 ml of 0.2N HCl. The solution was shaken for 20 min, and the water layer separated from the organic phase by centrifugation at 2500 rpm for 10 min was subjected to colorimetry at 355 nm. Plasma level of hydralazine was calculated by multiplying the optical density by a coefficient of 1.020.

Recently, Zak *et al.*¹³⁾ and Reece *et al.*¹⁴⁾ have reported that the hydralazine concentration in plasma determined according to the original

method of Zak *et al.*¹²⁾ actually includes acid-hydrolyzable hydralazine conjugates of unknown structure in addition to free hydralazine.

The data given here, therefore, represent "apparent" hydralazine level as originally reported by Zak *et al.*⁶⁾

RESULTS AND DISCUSSION

Effect of Hydralazine on the Tissue Glycogen Levels

Animals were decapitated 4 hr after hydralazine treatment (50 mg/kg, *i.p.*) or immediately after death, if any. Liver, diaphragm and heart were removed and glycogen levels of the tissues were determined as described in MATERIALS AND METHODS. As shown in Table I, hepatic and diaphragm glycogen levels were significantly decreased, while, cardiac glycogen increased.

Effect of Hydralazine Treatment on Blood Sugar Level

FIG. 2. Effect of Adrenalectomy on Hydralazine Induced Hyperglycemia in Rats

Eight intact rats (●) and 8 ADREX (○) were administered hydralazine (20 mg/kg, *i.p.*) and blood sugar level was determined up to 8 hr after the drug administration. Statistical significance between intact and ADREX were shown at each point. * $p < 0.001$. Details, see legend to Fig. 1.

FIG. 3. Effect of Pretreatment of Rats with Propranolol or Phentolamine on Hydralazine Induced Hyperglycemia

Intact rats responsive to hydralazine were pretreated with either propranolol (4 mg/kg, *i.p.*) (■) or phentolamine (20 mg/kg, *i.p.*) (△) 30 min before hydralazine treatment. Blood sugar level was determined up to 8 hr after the drug treatment. Figures in parentheses indicate number of animals used at each group. Statistical significance between control (●) and drug pretreated one was shown at each point.

* $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$.

Blood sugar level in rats was determined as a function of time up to 8 hr after hydralazine treatment. Figure 1a shows that blood sugar level was increased approximately twice the initial level 2 hr after hydralazine in male as well as female rats; thereafter, it began to decrease to initial level within 6 hr in females and 4 hr in males, respectively.

On the other hand, it is important to note that 6 out of 14 male and 5 out of 10 female rats tested were found to be non responsive (NR) to hydralazine-induced hyperglycemia (Fig. 1b). These NR rats were subjected to hydralazine treatment twice at 7-day intervals and similar results have been obtained. Although the reason of no response of some rats used to hydralazine-induced hyperglycemia remains obscure, individual difference in their ability to metabolize hydralazine may be involved.

Effect of Adrenalectomy on Hydralazine-induced

FIG. 4. Changes in cAMP Level of Plasma after Hydralazine Treatment in Intact Rats and ADREX

Intact male rats were administered hydralazine (○) and changes in blood cAMP level was compared to those in saline control (●) and in hydralazine-treated ADREX (■). Figures in parentheses indicate number of animals used in each group. * Significantly different from saline control ($p < 0.01$), ** significantly different from saline control ($p < 0.02$), † significantly different from ADREX ($p < 0.001$), †† significantly different from ADREX ($p < 0.05$).

Hyperglycemia in Rats

In order to ascertain the involvement of hormonal control by adrenals in hydralazine-induced hyperglycemia, adrenalectomized rats (ADREX) were used. As shown in Fig. 2, blood sugar level in ADREX remains unchanged by hydralazine treatment. This indicates that adrenal hormones seem to be closely related to the hyperglycemia.

Effect of Pretreatment of Rats with Propranolol or Phentolamine on Hydralazine-induced Hyperglycemia

Pretreatment of intact rats with propranolol, a β -adrenergic blocker, depressed almost completely the increase in blood sugar level after hydralazine treatment (Fig. 3). Furthermore,

FIG. 5. Time Course Studies on Plasma Level of Hydralazine in Male and Female Rats after Hydralazine Treatment

Figures in parentheses indicate number of animals used at each point. Each point represents mean $\pm$ S. E. (●), male; (○), female. * Significantly different from male ($p < 0.02$). ** significantly different from male ($p < 0.05$).

Figure 3 also shows that the hydralazine-induced hyperglycemia was depressed by pretreatment with phentolamine, an α -adrenergic blocker and an activator of cyclic nucleotides phosphodiesterase. These findings imply that cAMP may be involved in the hyperglycemia in this study.

Changes in Blood cAMP Levels in Rats after Hydralazine Treatment

CAMP levels of rat whole blood were determined up to 8 hr after hydralazine treatment. Figure 4 shows that cAMP level was significantly increased 30 min after hydralazine treatment, and gradually declined to initial level within 4 hr. In contrast, there is no significant increase in cAMP level in ADREX at any time after hydralazine treatment.

The Time Course Studies on Plasma Level of Hydralazine in Male and Female Rats after Hydralazine Treatment

Rats were administered hydralazine (20 mg/kg, *i.p.*) and the plasma level of hydralazine was determined up to 8 hr after the drug treatment. In this experiment the lower dose of hydralazine (20 mg/kg) was used because no rats will die at this dose. Figure 5 shows that there is a clear sex difference at the rate of decline of hydralazine level in plasma. This may explain the higher concentration of blood sugar in female rats after hydralazine treatment, as compared to that in male rats (Fig. 1).

In conclusion, the hyperglycemia in rats after hydralazine treatment may be due to the stimulation of adrenaline excretion from adrenals, leading to activation of glycogenolysis through cAMP pathway.

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