Effect of *Alpha*-2 Adrenergic Agonist on *Beta* Adrenoceptor-Mediated Control of Blood Glucose in the Fasted Mouse.

Guie In Han and Mie Young Kim
College of Pharmacy, Chung-Ang University, Seoul 151, *Korea*
(*Received November* 1, 1986)

Abstract □ Dose-dependent increases in blood glucose were produced by epinephrine and clonidine in fasted male mice. Isoproterenol was ineffective in increasing blood glucose at lower doses (10^{-8} M/kg—10^{-7} M/kg); with higher dose (10^{-6} M/kg) the glucose level was increased. The hyperglycemia induced by epinephrine was inhibited by yohimbine, prazosin and propranolol, indicating that the hyperglycemic effect of epinephrine is mediated by *alpha*-1, *alpha*-2 and *beta* adrenoceptor. When clonidine (10^{-6} M/kg) was administered simultaneously with isoproterenol (10^{-6} M/kg), an enhenced hyperglycemic effect was observed. The increment produced by clonidine plus isoproterenol was higher than that by clonidine alone. These results suggest that stimulation of *alpha*-2 adrenoceptor may be responsible for the exertion of the hyperglycemic effect by *beta* agonists in fasted mice.

Keywords □ Blood glucose, Hyperglycemia, Hypoglycemia, Epinephrine, Clonidine, Isoproterenol, Fasted mice, Adrenergic regulation of blood glucose.

It is generally accepted that the adrenergic mechanisms which regulate the hyperglycemic responses to catecholamine can be mediated by both *alpha* and *beta* receptors (1-3). Nevertheless, the adrenergic receptor mediating regulation of the plasma glucose level is a controversial subject. This is in part due to the differences in drug responses related to animal species and to the nutritional status of the animal (4-6). In addition, the hyperglycemia produced by adrenergic agonists *in vovo* is a complex resultant of several metabolic actions (7-9).

Thus the present study was carried out to furthur investigate the adrenergic regulation of plasma glucose level in fasted mice, using selected *alpha* and *beta* agonists and antagonists. Moreover, the effect of *alpha*-2 receptor stimulation in the hyperglycemic response to *beta* agonist was studied.

EXPERIMENTAL METHODS

Adult male ddY mice (20-25g), fasted for 15 hr, were used. Antagonists were given i.p. 30 min before agonists. Agonists were given s.c. and the animals were decaptiated 30 min after agonists. The blood was immediately collected and the plasma glucose was estimated by means of glucose oxidase method.

The drugs were dissolved in saline solution to the extent that a 0.1 m*l*/20g body weight volume could be administered.

Results were expressed as the mean±S.E.M. Statistical analyses were performed using the unpaired Student's *t* test.

The drugs used were obtained from: (—)-epinephrine bitartrate, clonidine hydrochloride, yohimbine hydrochloride and dl-propranolol hydrochloride, Sigma Chemical Company; prazosin hydrochloride, Pfizer; (±)-isoproterenol-hydrochloride, Tokyo Kasei.

RESULTS

Effects of alpha-and beta-adrenergic agonists on plama glucose level

Epinephrine and clonidine produced dose-related increases in blood glucose (Fig.1). The control glucose level was 108±4.2 mg/dl. In contrast, isoproterenol was ineffective in increasing blood glucose at lower doses (10^{-8} M/kg~10^{-7} M/kg); with higher dose (10^{-6} M/kg) the blood glucose level was

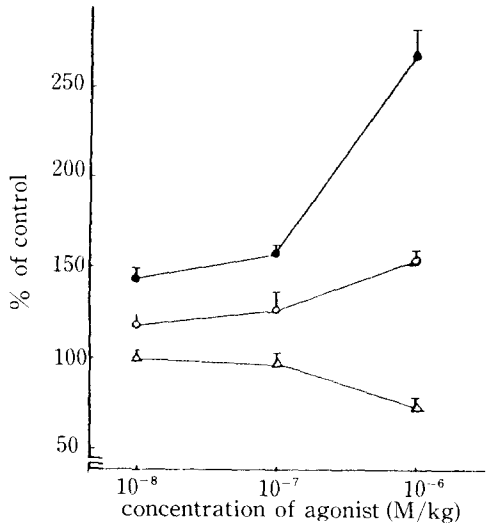

Fig.1. Effects of various adrenergic agonists, epinephrine (●), clonidine (○) and isoproterenol (△), on plasma glucose level.

Each point represents the mean±S.E.M. of at least 4 animals.

decreased.

Effects of various antagonists on alpha and beta agonist-induced hyperglycemia

The hyperglycemia induced by epinephrine was markedly inhibited by yohimbine, an *alpha*-2 antagonist, and propranolol, a *beta* antagonist (Fig.2). Prazosine, an *alpha*-1 antagonist, also

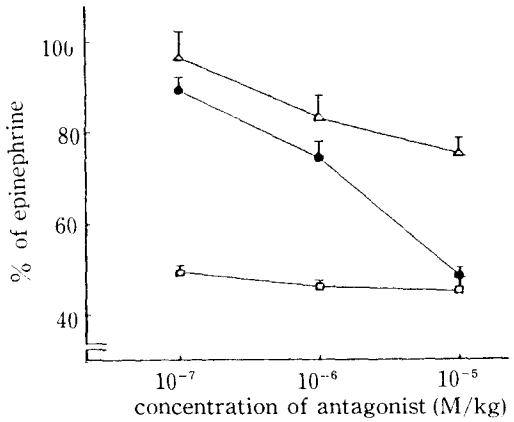

Fig.2. Effects of adrenergic antagonists on epinephrine-induced hyperglycemia.

△-△, prazosin, ●-●, yohimbine, □-□, propranolol. Antagonists were given 30 min before administration of epinephrine (10^{-6} M/kg). Each point represents the mean±S.E.M. of at least 4 animals.

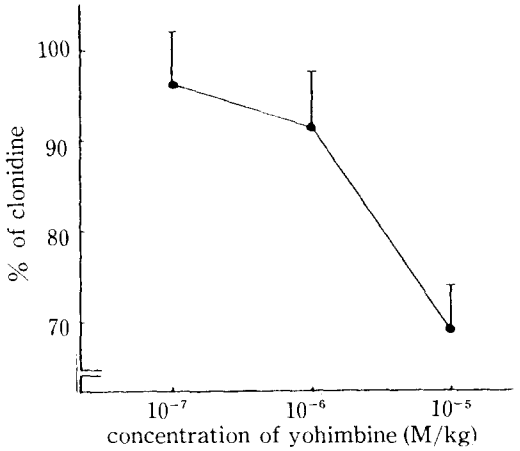

Fig.3. Effects of yohimbine on clonidine-induced hyperglycemia.

Yohimbine was given 30 min before administration of clonidine (10^{-6} M/kg). Each point represents the mean±S.E.M. of at least 4 animals.

inhibited the epinephrine-induced hyperglycemia, the degree of inhibition was weaker than that of yohimbine and propranolol. The results indicate that the hyperglycemic effect of epinephrine is mediated by *alpha*-1, *alpha*-2 and beta-adrenergic receptor.

The effect of clonidine was inhibited by yohim-

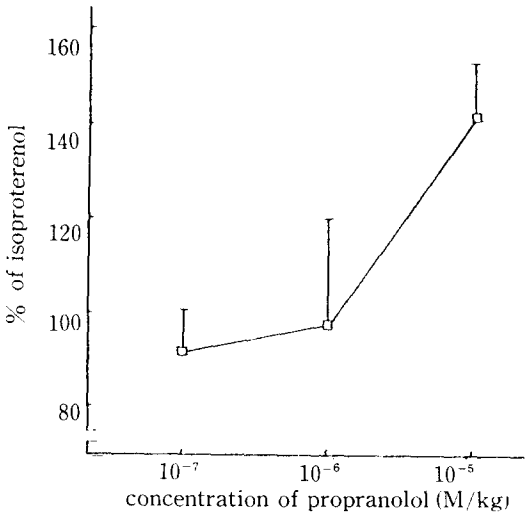

Fig.4. Effect of propranolol on isoproterenol-induced hypoglycemia.

Propranolol was given 30 min before administration of isoproterenol (10^{-6} M/kg). Each point represents the mean±S.E.M. of at least 4 animals.

bine (Fig.3). Thus, it is confirmed that the plasma glucose level can be increased by the effects of *alpha*-1, *alpha*-2 and *beta* adrenergic receptor.

Propranolol antagonized the hypoglycemic effect of isoproterenol, thus increased plasma glucose to the level which is slightly higher than control (Fig. 4). It should be noted that the beta adrenergic antagonist was very effective for inhibiting epinephrine-induced hyperglycemia (Fig.2). These results indicate that both as a *beta* agonist, epinephrine is a potent hyperglycemic agent while isoproterenol is not.

Effect of clonidine on isoproterenol-induced hypoglycemia

To assess the possible contribution of *alpha*-2 adrenergic mechamism to *beta* adrenergic mediated controlling of plasma glucose level, an *alpha*-2 adrenergic agonist, clonidine, was administered simultaneously with isoproterenol (Fig.5). Hypoglycemia produced by isoproterenol (10^{-6} M/kg) was reversed to hyperglycemia by clonidine (10^{-6} M/kg). The hyperglycemic effect of clonidine plus isoproterenol was higher in comparison with the effect of clonidine alone. This effect was suppressed by propranolol (10^{-5} M/kg). The hyperglycemic effect of clonidine alone was not affected by the antagonist (Fig.5).

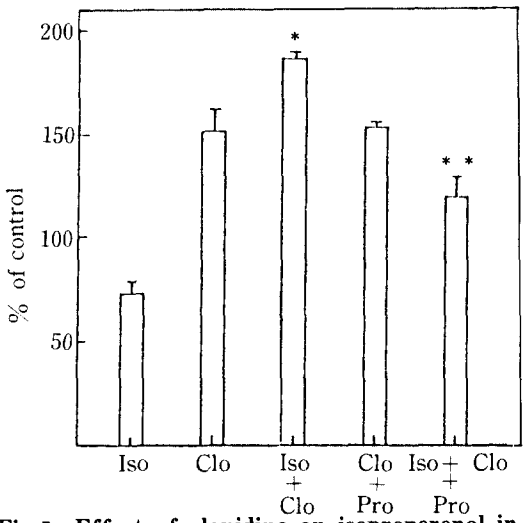

Fig.5. Effect of clonidine on isoproperenol-induced hypoglycemia and antagonism of its effect by propranolol.

Propranolol (10^{-5} M/kg) was given 30 min before administration of isoproterenol (10^{-6} M/kg) or isoproterenol (10^{-6} M/kg) plus clonidine (10^{-6} M/kg) or clonidine alone. Each result represents the mean±S.E.M. of at least 4 animals.

* Significantly different from clonidine alone ($p<0.01$)

** Significantly different from iso + clo ($p<0.01$)

DISCUSSION

The present results show that an increase in plasma glucose is produced by both *alpha* and *beta* agonists (Fig.1). Furthermore, the hyperglycemia induced by epinephrine was inhibited by prazosin, yohimbine and propranolol (Fig.2), indicating that *alpha*-1, *alpha*-2 and *beta* adrenergic receptors can mediate the hyperglycemic response to epinephrine.

On the other hand, isoproterenol, a *beta* agonist was ineffective in elevating plasma glucose at lower doses; at higher does, it decreased the plasma glucose level.

Earlier results relating to the hyperglycemic responses to the *beta* adrenergic agonists have brought some controvesy (3-6). It has been reported that there are characteristic species differences in *beta* agonist-induced hyperglycemia. In dog and cat, isoproterenol is a potent hyperglycemic agent comparable to epinephrine. In the other species such as rat and rabbit, isoproterenol is considerably less potent than epinephrine for increasing plasma glucose. In the present study with fasted mice, epinephrine produced a marked increase in plasma glucose, while isoproterenol produced no effect or reduced it. Therefore, we assessed the possible involvement of *alpha*-2 receptor stimulation in the differential effects of the *beta* agonists in the control of plasma glucose level, since epinephrine acts on both *alpha*-2 and *beta* adrenoceptor while isoproterenol acts mainly on *beta* adrenoceptor.

Clonidine, when used simultaneously with isoproterenol, modified the isoproterenol effect on plasma glucose level. The enhanced effect was shown by clonidine plus isoproterenol compared with the effect by clonidine alone. This combined effect of two agonists was inhibited by propranolol, a *beta* antagonist, which had no effect on clonidine. (Fig.5).

It is known that *beta* and *alpha*-2 receptors mediate various effects acting in opposite ways on adenylate cyclase activity and cAMP production (7, 10). Thus, it is expected that *alpha*-2 adrenergic receptor stimulation can be counteracted by *beta* agonists. Since the effect on the insulin secretion is considered to be the most important mechanism of *beta* agonist in reducing blood glucose (6,

11), and stimulation of *alpha*-2 adrenoceptor in pancreatic cell inhibits insuline release (2, 11), it could be explained that both the *alpha*-2 and the *beta* agonist act on insulin secretion in opposite way and the overeffect produced by the two agonists is hyperglycemia. However, other possibility may also exist. The results obtained show that the increase of blood glucose produced by clonidine plus isoproterenol was significantly higher than that produced by clonidine alone. Propranolol suppressed the enhanced effect only to the same extent of the level which is produced by clonidine alone. Thus, the predominant effect of *beta* agonist in fasted mice is hypoglycemia, and upon the stimulation of *alpha*-2 adrenoceptor, the hyperglycemic effect of *beta* adrenergic agonist may be unmasked.

LITERATURE CITED

1. Lum, K.B., Lau, Y.S., Buesa, R., Lockwood, R.H. and Kuo, S.H.: Studies on the hyperglycemia and hepatic glycogenolysis produced by *alpha* and *beta* adrenergic agonists in the cat. *Life Sci.*, **76**, 1195(1980).
2. Nakadate, T., Nakaki, T., Muraki, T. and Kato, R.: Adrenergic regulation of blood glucose levels: Possible involvement of postsynaptic *alpha*-2 type adrenergic receptors regulating insulin release. *J. Pharmacol. Exp. Ther.* **215**, 226(1980).
3. Arnold, A. and McAuliff, J.P.: *Alpha*-receptor mediated hyperglycemia in the mouse. *Arch. Int. Pharmacodyn. Ther.* **189**, 243(1971).
4. Ellis, S.: Some explanations for species differences in hyperglycemic responses to adrenergic agents. *Life Sci.* **22**, 1229(1978).
5. Kuo, S.H., Kamaka, J.K. and Lum, K.B.: Adrenergic receptor mechanisms involved in the hyperglycemia and hyperlacticacidemia produced by sympathomimetic amines in the cat. *J. Pharmacol. Exp. Ther.* **202**, 302(1977).
6. Poter, D.E. and Ellis, S.: Isoproterenol- and epinephrine-induced changes in blood glucose and tissue glycogen levels in normal and diabetic rats: The influence of alteration in endogenous insulin levels and state of nourishment. *J. Pharmacol. Exp. Ther.* **193**, 576(1975).
7. Exton, J.H.: Molecular mechanism involved in *alpha*-adrenergic responses. *Mol. Cell Endocrinol.* **23**, 233(1981).
8. Kunos, G.: The hepatic α-adrenoceptor. *Trends Pharmacol Sci.* **5**, 380(1984).
9. Himms-Hagen, J.: Sympathetic reglation of metabolism. *Pharmacol. Rev.* **19**, 367(1967).
10. Tran. M.A., Lac, D.T. Berlan, M. and Lafontan, M.: Interplay of *alpha*-2 and beta adrenoceptors in the control of free fatty acid release from bone marrow adipose tissue. *J. Pharmacol. Exp. Ther.* **230**, 226(1984).
11. Young, J.B. and Landsberg, L.: Catecholamines and the regulation of hormone secretion. *Clin Endocrinol. Metb.* **6**, 657(1977).