Isoproterenol and Epinephrine-Induced Hyperglycemias in Rabbits: Effects of Alloxan Treatment and Prandial State (36339)

DAVID E. POTTER, JULIO MORATINOS, AND SYDNEY ELLIS

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550

Recently, it was reported from this laboratory that *l*-isoproterenol is more potent than *l*-epinephrine in releasing glucose from rabbit liver slices (1). In contrast, dose–response data in unanesthetized rabbits have shown that *l*-epinephrine is more potent than *d,l*- or *l*-isoproterenol in elevating blood glucose when administered by subcutaneous injection (2–4) or by intravenous infusion (4). Differences in potency *in vivo* and *in vitro* may be due to differing activity upon the liver and/or to indirect actions of catecholamines as a consequence of effects on other organs. Secretion of insulin appears to be an important mechanism for the influence of catecholamines on carbohydrate metabolism. Observations in man, in laboratory animals, and in isolated pancreatic tissue have shown that isoproterenol is capable of promoting insulin release, whereas epinephrine usually inhibits insulin release (5–10). More recently it has been suggested that isoproterenol-induced insulin release in some animal species may be a function of a β-2 type adrenergic receptor (11).

Methods. In view of the complex interactions which may occur *in vivo*, experiments were designed to test for a possible influence of insulin release on catecholamine-induced hyperglycemia. White New Zealand male rabbits were divided into three groups and treated as follows: fasted rabbits (group I) were deprived of food for 24 hr prior to the experiment, but had free access to water; fed rabbits (group II) were allowed free access to food and water; diabetic-fed rabbits (group III) were allowed free access to food and water. Diabetes was produced by an intravenous infusion with 150 mg/kg of alloxan monohydrate over a 10–20 min period into rabbits with access to food before and after the injection (12). To prevent the development of fatal hypoglycemia during the 12 hr subsequent to the alloxan administration, each rabbit was given 5–10 ml of 25% glucose solution subcutaneously at 2–3 hr intervals. Alloxan treatment was considered successful if plasma glucose levels were more than 300 mg/100 ml on days 2 and 3 after alloxan was administered. Rabbits were allowed about 14 days for recovery following alloxan treatment; and, during this period, the appropriate controlling dose of protamine zinc insulin (PZI) was determined. The daily dose of PZI (6–12 units/animal) required to control the blood glucose level was given subcutaneously, late in the afternoon. The regimen of alloxan treatment followed by replacement therapy with insulin provided the rabbits with basal levels of insulin for control of blood glucose levels, but limited the availability of endogenous insulin for acute release. The criterion for selection of an individual alloxan-treated, insulin-controlled rabbit for inclusion in this study was a plasma glucose level of 120–200 mg/100 ml.

Blood samples for glucose analysis were removed through an indwelling cannula in the central artery of the ear. Intravenous infusions of drugs were made via a cannula placed in the marginal vein of the contralateral ear. After removal of two control blood samples separated by an interval of 30 min, an intravenous infusion of saline or drug solution was given at a constant rate of 0.2 ml/min for 30 min. Additional samples of blood were removed at designated intervals during and after the infusion. Separated, heparinized plasma was analyzed for glucose by the Hoffman method (13) as adapted for

TABLE I. Effects of Prandial State and of Alloxan Treatment and Insulin Replacement Therapy on Tissue Glycogen Content and Blood Glucose Levels in Rabbits.

Treatment	No.	Tissue glycogen (mg/g of wet wt)		Plasma glucose (mg/100 ml)
		Liver	Muscle	
Normal, fasted	11	4.9 ± 0.7[a,b]	3.2 ± 0.3	115 ± 2.9[c]
fed	5	15.8 ± 2.3	3.3 ± 0.4	155 ± 17.2
Alloxan-treated, insulin-controlled, fed	5	16.1 ± 2.0	4.1 ± 0.9	156 ± 11.6

[a] Mean ± standard error.

[b] Significance of difference between values from fasting and nonfasting animals: $p < .001$; [c] $p < .05$.

the Technicon AutoAnalyzer. Concentrations of drugs are expressed in terms of the active component.

In some saline-treated animals from each group, samples of muscle and liver were taken (under pentobarbital anesthesia) and analyzed for glycogen concentration (14, 15).

Results. The suitability of treatment with alloxan and the subsequent replacement therapy with PZI was supported by gross observation of the animals as to general health and by measurements of tissue glycogen and plasma glucose levels. Table I shows the liver and muscle glycogen levels, as well as plasma glucose concentrations in saline-treated animals from each group. Liver glycogen content and blood glucose levels were significantly lower in fasted rabbits than in fed rabbits, whereas muscle glycogen content was similar in both groups. Alloxan-treated, insulin-controlled rabbits (referred to below as diabetic-fed rabbits) had tissue glycogen levels that were not significantly different from those of normal-fed rabbits, although muscle glycogen levels were insignificantly higher in diabetic animals. Basal levels of plasma glucose in normal-fed and diabetic-fed rabbits were virtually the same.

In normal rabbits intravenous infusions of isoproterenol (30 μg kg^{-1} min^{-1}) produced hyperglycemic responses of similar magnitude whether the rabbits were fed or fasted,

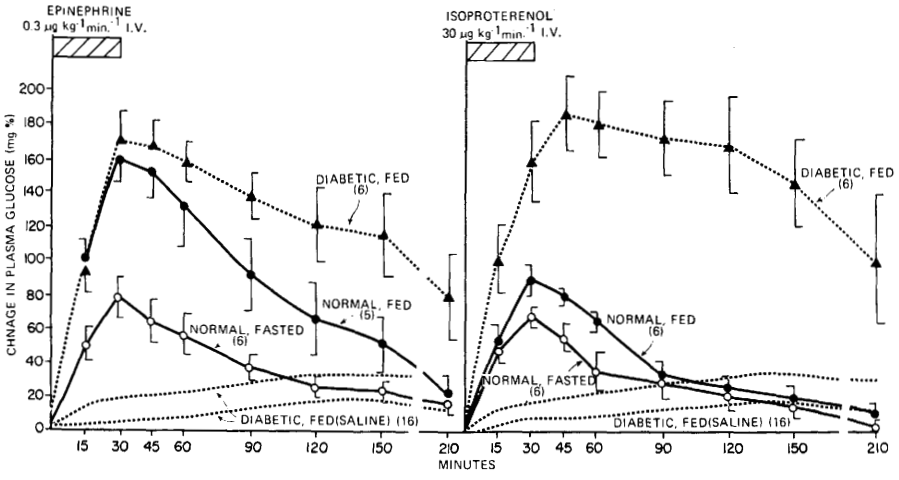

FIG. 1. Changes in plasma glucose levels induced by epinephrine and isoproterenol: (each point) the mean response of the number of rabbits shown in parentheses; (brackets on each point) the standard error; (- - - -; parallel) the range of the mean ± SE for saline-treated rabbits.

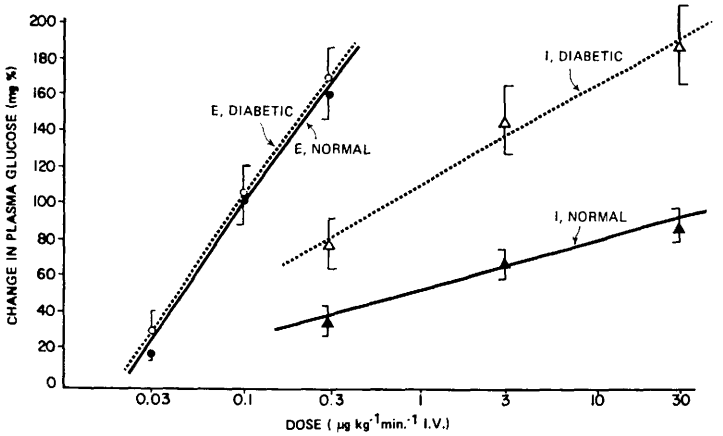

FIG. 2. Dose–response curves to epinephrine (E) and isoproterenol (I) in normal-fed and alloxan-diabetic, fed rabbits: (points and limits on each point) the mean peak response and standard error, respectively. The difference is significant ($p < .02$) between normal-fed and alloxan-diabetic, fed rabbits at each concentration of isoproterenol.

whereas infusions of epinephrine (0.3 μg kg^{-1} min^{-1}) produced greater responses in fed, than in fasted rabbits (Fig. 1). Diabetic-fed rabbits, however, showed a much greater hyperglycemic response to the same concentration of isoproterenol than did normal-fed rabbits (Fig. 1). Epinephrine, on the other hand, produced about the same elevation of blood glucose in normal-fed as in diabetic-fed rabbits (Fig. 1). The control of alloxan-diabetic rabbits with insulin over the experimental period is evident from the small rise in blood glucose when these animals were infused with isotonic saline, 0.2 ml kg^{-1} min^{-1}. Figure 2 summarizes the dose-related responses obtained with epinephrine and isoproterenol in normal-fed rabbits and in diabetic-fed rabbits. The epinephrine dose–response curves for both groups of rabbits are similar and practically superimposed. For each concentration of isoproterenol tested, the hyperglycemic response in alloxan-treated animals was approximately 2–2.5 times greater than that demonstrated by normal-fed rabbits; in terms of potency the activity of isoproterenol was increased 10–100 times.

The increased hyperglycemic activity of epinephrine in normal-fed rabbits over that observed in normal-fasted rabbits is probably related to increased hepatic glycogen levels in the fed state (Table I). Isoproterenol did not produce an increased hyperglycemic response in normal-fed rabbits as one might predict based on the *in vitro* activity of isoproterenol on liver slices reported by Mülbachova *et al.* [1972]. From our results with alloxan pretreatment and prandial manipulation, it is suggested that the hyperglycemic activity of isoproterenol is modified in normal rabbits by a concomitant release of insulin. This proposal is supported by the enhanced hyperglycemic activity of isoproterenol in alloxan-treated rabbits and by the absence of an increased glycemic response in the presence of increased hepatic glycogen levels when normal-fed rabbits are compared with normal-fasted rabbits. The lack of increased glycemic response to epinephrine in alloxan-treated animals is compatible with the evidence that this catecholamine inhibits the release of insulin in normal rabbits.

Summary. 1. The hyperglycemic effects of epinephrine and isoproterenol were compared in rabbits subjected to changes in prandial state and to treatment with alloxan.

2. Hyperglycemic responses to epinephrine were greater in normal-fed rabbits than in normal-fasted rabbits. Changes in the prandial state produced no alteration in the hyperglycemic response to isoproterenol.

3. Isoproterenol produced greater hyperglycemic responses in alloxan-diabetic, fed

rabbits than in normal-fed rabbits, whereas, epinephine was of equal potency in alloxan-diabetic, fed rabbits and normal-fed rabbits.

4. The marked difference in the relative potencies of isoproterenol and epinephrine in elevating blood glucose (EPI > ISO) in normal rabbits and in increasing glucose release from rabbit liver slices (ISO > EPI) may be related, at least in part, to the insulin-releasing activity of isoproterenol and to the suppression of insulin release by epinephrine.

The skillful technical assistance of Miss Lois Wilson in these experiments is gratefully acknowledged. The authors also wish to express their appreciation to Dr. Mary A. Root of Eli Lilly and Company for a generous gift of protamine zinc insulin, and to Mrs. Dorothy Austin for her secretarial assistance.

This work was supported in part by NIH Grant NS 07700 05.

1. Mühlbachova, E., Fed. Proc.. Fed. Amer. Soc. Exp. Biol. **28**, 1705 (1969); Mühlbachova, E., Chan, P. S., and Ellis, S., J. Pharmacol. Exp. Ther., in press (1972).

2. McChesney, E. W., McAuliff, J. P., and Blumberg, H., Proc. Soc. Exp. Biol. Med. **71**, 220 (1949).

3. Chen, G., Portman, R., Russell, D., and Ensor, C., J. Amer. Pharm. Ass. **40**, 273 (1951).

4. Potter, D. E., and Moratinos, J., Fed. Proc. **30**, 1684 (1971).

5. Porte, D., Jr., Diabetes **15**, 543 (1966).

6. Porte, D., Jr., Diabetes **16**, 150 (1967).

7. Malaisse, W. J., Malaisse-Lagae, F., Wright, P. H., and Ashmore, J., Endocrinology **80**, 975 (1967).

8. Majid, P. A., Saxton, C., Sykes, J. R. W., Galvin, M. C., and Taylor, S. H., Brit. Med. J. **4**, 328 (1970).

9. Loubatieres, A., and Mariani, M. M., C. R. Acad. Sci. (Paris) **270**, 3321 (1970).

10. Burr, I. M., Balant, L., Stauffacher, W., and Renold, A. E., Europ. J. Clin. Invest. **1**, 216 (1971).

11. Loubatieres, A., and Mariani, M. M., C. R. Acad. Sci. (Paris) **271**, 1046 (1970).

12. Pincus, I. J., Horwitz, J. J., and Scott, M. E., Proc. Soc. Exp. Biol. Med. **86**, 553 (1954).

13. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).

14. Good, C. G., Kramer, H., and Somogyi, M., J. Biol. Chem. **100**, 485 (1933).

15. Nelson, N., J. Biol. Chem. **153**, 375 (1944).

Received Dec. 2, 1971. P.S.E.B.M., 1972, Vol. 139.