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Changes in Plasma Glucose and Insulin Levels Induced by Adrenergic Agents in Normal and Alloxan-Diabetic Rats (39666)

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The limited ability of isoproterenol to elevate blood glucose levels in normal rats is well established (1-3). Previous experiments from this laboratory have indicated that the effects of isoproterenol on the pancreas can override the direct effect of this and other hyperglycemic agents on the liver (4). Elimination of the β cells of the pancreas has been demonstrated to augment markedly the hyperglycemic activity of isoproterenol (5) and to reverse the suppression of epinephrine-induced hyperglycemia by isoproterenol (4).

The experiments to be described were designed to determine the likelihood that isoproterenol-induced insulin release and its consequences in normal rats as well as isoproterenol-induced hyperglycemia in alloxan diabetic rats are the results of stimulating β-adrenergic receptors. Propranolol, a potent β-adrenergic receptor antagonist, was used to examine these possibilities. In addition, the effects of epinephrine and a more selective β-adrenergic drug salbutamol on plasma insulin and glucose levels were also studied. Isoproterenol, salbutamol, epinephrine and norepinephrine were also compared for hyperglycemic activity in alloxan diabetic rats controlled with insulin.

Methods and materials. Male rats weighing approximately 250 g were obtained from Holtzman Rat Company (Madison, Wisconsin). All animals had free access to Purina Rat Chow and water until the time of the experimental procedure. Alloxan diabetes was induced by a procedure previously described (5).

In experiments examining the effect of isoproterenol and propranolol on plasma insulin and glucose levels, 10 mg/kg propranolol was administered ip 30 min prior to an injection of isoproterenol, 1 mg/kg ip or of acidified saline. Animals were lightly anesthetized with pentobarbital (30 mg/kg ip) just before taking blood samples by cardiac puncture. Thirty-five minutes elapsed between the time of injection of isoproterenol and sampling by cardiac puncture. Heparinized blood samples were centrifuged immediately. The plasma was separated and either analyzed immediately or frozen for analysis at a later date (no longer than 2 weeks).

In another set of experiments, epinephrine and salbutamol were examined for their ability to influence plasma insulin and glucose levels in rats. The method of anesthesia required for cardiac sampling and the handling of samples were the same as alluded to previously.

Experiments were also conducted to compare the hyperglycemic activities of isoproterenol, salbutamol, epinephrine and norepinephrine in normal rats and in diabetic rats. Blood samples (100 μl) for glucose determination were obtained by cutting the tip of the tail just before and one hour after ip injection of drugs.

Plasma levels of immunoreactive insulin were determined by means of a modification of the radioimmunoassay procedure described by Hales and Randle (6); insulin binding reagent (Burroughs-Wellcome Co., Research Triangle Park, N.C.) was used instead of individual insulin antibody and precipitating antibody. Glucose levels were determined by the Hoffman procedure (7) as adapted to the Technicon autoanalyzer.

Acidified stock solutions (pH 5) of l-isoproterenol, l-epinephrine, and l-norepinephrine bitartrate were diluted with physiologic saline to appropriate concentrations for injection. Two or three drops of acetic acid were used to enhance the solubility of salbutamol in saline.

Results. As shown in Fig. 1 (left panel) isoproterenol, 1 mg/kg (base) ip caused a

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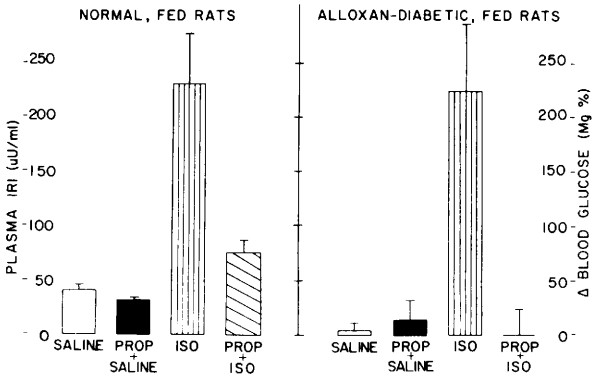

FIG. 1. Effect of pretreatment with propranolol on isoproterenol-induced insulin release in fed normal rats (left panel) and hyperglycemia in fed alloxan-diabetic rats maintained with daily injections of protamine zinc insulin (right panel). The mean control levels for blood glucose in the diabetic groups (right panel) were: 176±30, 160±50, 163±40, and 176±35, respectively. Each column represents the mean ±SE of six rats. The differences between the ISO group and PROP+ISO group in each panel are significant, $P < 0.001$.

marked rise in peripheral levels of immunoreactive insulin at 45 min after injection in fed normal rats. This hyperinsulinemia was accompanied by little change in blood glucose levels (<20 mg/100 ml; see Table II). A 10 mg/kg ip injection of propranolol followed by an injection of saline produced no change in insulin from control. However, pretreatment with propranolol suppressed the isoproterenol-induced change in insulin levels completely (left panel, Fig. 1).

Figure 1 (right panel) summarizes the effects of propranolol and isoproterenol alone and in combination on blood glucose levels in fed alloxan-diabetic insulin-treated rats. Isoproterenol alone produed little or no rise in blood glucose levels in fed normal rats as previously reported (4) and shown in Table II. Interestingly, in rats with few or no functioning pancreatic *beta* cells, isoproterenol became a very potent hyperglycemic agent. The maximum change in blood glucose level produced by 1 mg/kg of isoproterenol in fed, alloxan-treated rats was about 215 mg%. Pretreatment with propranolol eliminated completely the hyperglycemic effect of isoproterenol in diabetic animals. Treatment with propranolol followed by a sham injection of saline did not markedly influence the basal blood glucose levels of diabetic rats.

In another set of experiments the effects of salbutamol and epinephrine on plasma levels of immunoreactive insulin and glucose were investigated in fed, normal rats. As shown in Table I, salbutamol proved to be a more active hyperglycemic agent than isoproterenol but less active than epinephrine in fed normal rats. On the other hand, isoproterenol appeared to be the more potent of the two *beta* agonists in terms of insulin release; epinephrine was inactive. Salbutamol produced changes of 18, 34, and 95 mg/100 ml in plasma glucose at 0.01, 0.1, and 1 mg/kg ip. Significant elevations ($P < 0.05$) in plasma IRI levels were observed following 0.01 and 1 mg/kg of salbutamol, however, a significant elevation in the insulin/glucose ratio occurred only with the intermediate (0.1 mg/kg) dose (0.38 compared to 0.23 with saline). By way of comparison and as shown in Table I, 0.1 mg/kg of isoproterenol produced an insulin/glucose ratio of 0.67 compared to a value of 0.20 in a paired saline-treated group.

A comparison of the hyperglycemic effects of the adrenergic drugs (Table II) in normal and diabetic rats demonstrated that the activity of isoproterenol changed considerably more than that of salbutamol (only one dose studied in diabetic rats), epinephrine or norepinephrine when insulin release was suppressed. Thus, the variation in blood glucose levels produced by isoproterenol in diabetic rats is most probably a reflection of the degree of suppression of insulin release.

Discussion. The results presented in this paper are in general agreement with the concept that β-adrenergic agonists stimulate

TABLE I. THE EFFECTS OF ISOPROTERENOL, SALBUTAMOL AND EPINEPHRINE ON PLASMA IMMUNOREACTIVE (IRI) INSULIN LEVELS AND PLASMA GLUCOSE LEVELS IN FED NORMAL RATS.

Drug and dose (mg/kg ip)	Plasma IRI (μU/ml)	Plasma Glucose (mg/100 ml)	Ratio IRI/Glucose
Saline (6)[a]	39 ± 5[b]	156 ± 8	0.20 ± 0.02
Isoproterenol (base)			
0.01 (7)	89 ± 20[c]	174 ± 9	0.49 ± 0.10[c]
0.1 (7)	110 ± 19[d]	166 ± 11	0.67 ± 0.15[d]
1.0 (5)	227 ± 44[d]	160 ± 8	1.57 ± 0.29[d]
Saline (6)	31 ± 4	139 ± 4	0.23 ± 0.02
Salbutamol (base)			
0.01 (3)	24 ± 3	157 ± 10	0.16 ± 0.02
0.1 (4)	63 ± 8[c]	173 ± 6[c]	0.38 ± 0.04[c]
1.0 (5)	57 ± 9[c]	234 ± 9[d]	0.24 ± 0.04
Epinephrine (base)			
0.3 (8)	23 ± 4	381 ± 40[d]	0.06 ± 0.01[d]

[a] Number of animals given in parentheses.
[b] Mean ± SE (Students t test used to determine probability values).
[c] $P < 0.05$ Significantly different from control (saline).
[d] $P < 0.01$ Significantly different from control (saline).

TABLE II. CHANGES IN BLOOD GLUCOSE LEVELS INDUCED BY ISOPROTERENOL, SALBUTAMOL, EPINEPHRINE AND NOREPINEPHRINE IN FED NORMAL RATS AND FED ALLOXAN DIABETIC RATS CONTROLLED WITH INSULIN.

Drug and dose (mg/kg ip)	Change in blood glucose[a] (mg/100 ml)	
	Normal rats[b]	Alloxan-treated rats
Saline (5)	17 ± 8	−13 ± 10
Isoproterenol (base)		
0.01 (6)[c]	18 ± 5	57 ± 22[d]
0.1 (6)	10 ± 3	100 ± 9[e]
1.0 (6)	8 ± 4	222 ± 60[f]
Salbutamol (base)		
0.01 (3)	18 ± 10	
0.1 (4)	34 ± 6	59 ± 9[d]
1.0 (5)	95 ± 13	
Epinephrine (base)		
0.03 (4)	13 ± 9	1 ± 9
0.1 (7)	49 ± 11	53 ± 15
0.3 (10)	135 ± 12	99 ± 19[d]
Norepinephrine (base)		
0.1 (6)	22 ± 4	9 ± 17
0.3 (5)	46 ± 12	21 ± 26
1.0 (6)	63 ± 4	71 ± 26

[a] Blood taken from tail 60 min after drug injection.
[b] Fed normal rats serving as controls for comparison with diabetic rats.
[c] Number of animals given in parentheses.
[d] $P < 0.05$.
[e] $P < 0.01$.
[f] $P < 0.001$.

insulin release in the rat, and that α-adrenergic agonists inhibit release (4, 8, 9). Moreover, the data presented here would strongly suggest that isoproterenol has greater insulin releasing activity than does salbutamol or epinephrine in the fed normal rat. This suggestion is supported by the evidence that salbutamol and epinephrine have greater hyperglycemic to insulin releasing activity than isoproterenol in the fed normal rats as emphasized by their lower insulin/glucose ratios.

The difference in the metabolic activity between salbutamol and isoproterenol may be attributable in part to the relative rates of metabolic inactivation, salbutamol being inactivated somewhat more slowly than isoproterenol by virtue of its *meta*-CH_2OH (10). In addition, and perhaps more importantly, there may be significant differences in the selectivity of receptor activation between different target organs. Salbutamol is reported to be more of a selective β-adrenergic agonist for receptors in the bronchioles (β_2) than in the heart (β_1) (12). From this study, one might suggest that there is also a difference in selectivity and/or activity at the level of the liver and the pancreas. For example, salbutamol seemed to have a lesser effect on insulin release than isoproterenol and hence, salbutamol was a more active hyperglycemic agent than isoproterenol in normal rats and in normal rabbits (12). Moreover, the insulin-releasing activity of salbutamol may be due in part to its hyperglycemic effect.

It has been demonstrated that isoproterenol evokes dose-related increases in plasma insulin levels in the rat and that these elevations in insulin are strongly influenced by

the state of nourishment (4). Presumably isoproterenol is a weaker hyperglycemic agent in the fed compared to the fasted rat by virtue of its propensity to raise insulin levels more in the fully nourished state. Suppression of insulin released by alloxan treatment was shown to augment the glucose releasing activity of isoproterenol (5) more strongly than that of salbutamol, epinephrine and norepinephrine. That this augmented hyperglycemic effect of isoproterenol in the alloxan diabetic rat is the result of β-receptor stimulation, was demonstrated by the blockade of isoproterenol-induced hyperglycemia by pretreatment with propranolol. Furthermore, the elevations in plasma immunoreactive insulin produced by isoproterenol were also markedly suppressed by previous treatment with the *beta* receptor antagonist, propranolol.

Summary. Isoproterenol-induced insulin release in fed normal rats and hyperglycemia in fed alloxan diabetic rats were inhibited by pretreatment with propranolol. Moreover, salbutamol, another β-adrenergic stimulant, appears to have some insulin releasing activity in the fed normal rat although its glucose mobilizing activity seems to be preponderant. Epinephrine and norepinephrine, on the other hand, have weak or no insulin releasing activity acutely and are more potent hyperglycemic agents than isoproterenol in the fed normal rat. These results suggest that insulin release by isoproterenol and by salbutamol in normal rats can attenuate the hyperglycemic activity of these β-adrenergic receptor agonists. Suppression of insulin release by treatment with alloxan discloses the substantial glucose mobilizing effects of isoproterenol in the rat.

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