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METABOLIC RESPONSES TO ISOPROTERENOL AND EPINEPHRINE IN THE RABBIT.

INFLUENCE OF STATE OF NOURISHMENT, ALLOXAN DIABETES AND PRETREATMENT WITH PROPRANOLOL*

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Abstract—Previous reports have shown that isoproterenol (ISO) is less active then epinephrine (EPI) in producing hyperglycemia in rabbits *in vivo* but ISO *in vitro* is more active than EPI in releasing glucose from rabbit liver slices. This difference in activities of the catecholamines *in vivo* was investigated by comparing responses in plasma glucose, lactate and glycerol in fed normal, fasted normal and fed alloxan-diabetic rabbits. Changes in the state of nourishment had more effect on the plasma glucose response to EPI than to ISO. In contrast, the hyperglycemic response to ISO was augmented by prior chemical destruction of pancreatic β-cells whereas the peak response to EPI was unchanged. Neither the lactate nor the glycerol responses to the catecholamines were modified drastically by changes in the state of nourishment or by lack of endogenous insulin release. Pretreatment with propranolol in fed normal rabbits suppressed the rise in lactate and glycerol induced by both catecholamines, but the rise in glucose produced by EPI was not depressed and the rise produced by ISO was inhibited by only 50 per cent. These results suggest that the hyperglycemic response to EPI is primarily a result of hepatic glycogen breakdown whereas the response to ISO is largely dependent upon gluconeogenic mechanisms. Therefore, catecholamines may not only affect certain metabolic substrate levels by direct actions on liver, muscle and adipose tissue, but they may also influence the responsiveness of the pancreatic islet cells, which, in turn, alters the disposition of key metabolic substrates.

A previous paper from this laboratory has confirmed the greater hyperglycemic potency of epinephrine (EPI) as compared to isoproterenol (ISO) in fasted unanesthetized rabbits [1]. These data have also indicated that EPI-induced glycogenolysis in liver appears to be the primary mechanism by which the rise in blood glucose is produced. On the other hand, the release of lactate from muscle and glycerol from adipose tissue and their subsequent conversion to glucose in the liver could account for the moderate hyperglycemia evoked by ISO without concurrent reductions in liver glycogen content.

The present investigations were carried out to explore in more detail the responsiveness of muscle and adipose tissue to EPI and ISO in the rabbit after changes in the state of nourishment and elimination of acute insulin release by treatment with alloxan. Changes in the levels of metabolic substrate in response to catecholamines in the fed normal and fed diabetic rabbits should reflect the importance of acute insulin release as a controlling factor in the regulation of glucose, glycerol and lactate levels. In addition, the effect of beta-receptor blockade with propranolol was tested against the metabolic effects of EPI and ISO in fed normal rabbits.

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METHODS

Unanesthetized white New Zealand male rabbits weighing between 2 and 3.5 kg were used in all experiments after being conditioned for restraint in well-ventilated boxes. The rabbits were divided into three groups and subjected to the following conditions: group I, normal rabbits deprived of food only for 24 hr; group II, normal rabbits allowed free access to food and water; group III, alloxan-diabetic, insulin-controlled rabbits allowed food and water *ad lib.* Production of the diabetic state with alloxan and treatment with insulin are described in detail in a previous paper [2].

Serial blood samples were removed from the central artery of one ear and drugs were infused intravenously into the marginal vein of the contralateral ear. Blood samples were kept in an ice bath before and after centrifugation for separation of plasma. The arterial cannulae were kept patent by the slow infusion of physiological saline solution.

Plasma glucose levels were determined with a Technicon autoanalyzer which makes use of a modification of the procedure of Hoffman [3]. Blood lactate levels were quantified enzymatically using lactate dehydrogenase and measuring the amount of NADH formed spectrophotometrically at 340 nm [4]. Lactate levels were calculated according to the formula: maximum $O.D._{340} \times 65.5 = mg/100\,ml$ of lactate. The determination of glycerol involved the conversion of glycerol to lactate via a three-step enzymatic process

Table 1. Control values for plasma glucose, lactate and glycerol in rabbits

	State of rabbit	Glucose (mg/100 ml)	Lactate (mg/100 ml)	Glycerol (mg/100 ml)
Group A	Fasted normal N = 18	118 ± 5	11.3 ± 1.1	2.5 ± 0.2
Group B	Fed normal N = 15	140 ± 11*	8.7 ± 1.4	1.9 ± 0.2*
Group C	Fed, alloxan-diabetic, insulin-treated N = 15	164 ± 19*	10.2 ± 1.5	2.1 ± 0.2

* Significantly different from group A, $P < 0.05$.

involving glycerokinase, pyruvate kinase and lactate dehydrogenase [5]. The amount of NADH consumed during the conversion of pyruvate to lactate was measured spectrophotometrically at 366 nm. Free glycerol levels were calculated according to a predetermined formula: Δ O.D.$_{366} \times 17.5 =$ mg/100 ml of free glycerol.

The doses of catecholamines used in this study, 0.3 μg/kg/min of *l*-epinephrine and 30 μg/kg/min of *l*-isoproterenol, were chosen because they produced hyperglycemia of similar magnitude in fasted rabbits.

In order to examine the effects of beta-receptor blockade on metabolic responses to the catecholamines, propranolol was infused i.v. at a rate of 0.3 mg/kg/min for 30 min prior to the infusion with the catecholamines.

Stock solutions of the catecholamines were prepared in acidified saline (pH = 4.5) from their respective salts, hydrochloride for *l*-isoproterenol and bitartrate for *l*-epinephrine. Propranolol was available as the hydrochloride salt. All drug concentrations are expressed in terms of the free base.

Statistical comparisons were made using Student's *t*-test for paired or non-paired data [6].

RESULTS

Control levels of plasma glucose, lactate and glycerol. Table 1 lists the control values for glucose, lactate and glycerol levels based upon the state of nourishment and/or the state of function of the β-cells of the pancreas. The plasma glucose level of fasted normal rabbits was significantly lower than that of fed normal or fed diabetic rabbits treated with insulin. However, there was no significant difference between the glucose levels of fed normal and fed diabetic rabbits. A previous report from this laboratory has shown that tissue glycogen levels were lower in fasted normal rabbits whereas there were no apparent differences between fed normal and fed diabetic rabbits [2].

Control lactate levels in the fasted normal group were higher than in either fed group but the differences in levels were not statistically significant.

Plasma glycerol levels were significantly lower in the fed normal groups as compared to the fasted normal group. On the other hand there was not a statistically significant difference between the glycerol levels of the fasted normal and the fed diabetic groups of rabbits.

These control data may be interpreted as indicating that the fed diabetic insulin-treated group represents a metabolic intermediate somewhere between the fed normal and fasted normal groups of rabbits.

Changes in plasma glucose levels. Figure 1, panel A, summarizes the dose-related changes in plasma glucose levels produced by EPI and ISO. Previous studies had shown that, at all concentrations tested, the oustanding feature was the greater hyperglycemic response to EPI as compared to ISO in both the fasted and the fed states. Note that the peak hyperglycemic response to ISO was not changed significantly by fasting and feeding.

Panel A of Fig. 1 also compares the peak hyperglycemic responses of EPI and ISO in normal fed rabbits and in diabetic fed rabbits. At all doses represented, the highest responses induced by ISO in diabetic fed rabbits were twice as great as those in normal fed rabbits. On the other hand, EPI produced hyperglycemic responses in the two groups of fed rabbits that were of similar magnitude.

Previous results from this laboratory have demonstrated that a 24 hr fast affects primarily the liver glycogen content with little effect on muscle glycogen levels [7]. Furthermore, no significant difference was observed between the liver glycogen levels of normal fed rabbits and alloxan-diabetic, insulin-controlled rabbits in the fed state.

Changes in plasma glycerol levels. In the evaluation of the responsiveness of adipose tissue, the doses of EPI and ISO utilized where those producing quantitatively similar hyperglycemic responses in normal fasted rabbits. As shown in Fig. 1, panel B, basal glycerol levels in saline-treated rabbits were slightly higher in fasted than in fed rabbits. The elevation in glycerol levels produced by ISO was not significantly changed by feeding and fasting, but the response to EPI was significantly greater in the fed group ($P < 0.05$) than in the fasted group when the basal levels of the two groups are taken into consideration. It is highly probable that the dose of ISO (30 μg/kg/min, i.v.) used is producing a near maximal response in all groups.

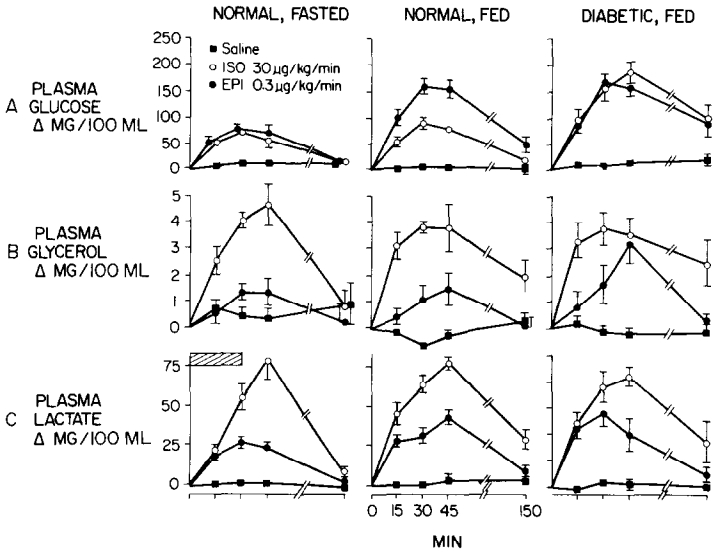

Fig. 1. Changes in metabolic substrate levels produced by i.v. infusions of *l*-isoproterenol, *l*-epinephrine and saline. Diabetic rabbits were maintained with protamine zinc insulin throughout the course of these experiments. Drugs were infused over a 30-min period. Each point represents the mean ± S. E. of determinations in each of six animals.

In the diabetic fed rabbits EPI produced a significantly greater increase in glycerol levels at 45 min than occurred in normal fed rabbits (3.4 ± 0.4 vs 1.5 ± 0.6 mg/100 ml, $P < 0.05$). No significant difference could be detected between the maximal changes in glycerol evoked by ISO in the normal and diabetic groups.

Changes in plasma lactate levels. Plasma lactate levels were virtually the same in all three groups of rabbits infused with 0.9% saline (Fig. 1, panel C). Again, ISO (30 μg/kg/min) produced marked increases in plasma lactate levels regardless of the state of nourishment or state of pancreatic function. No significant difference could be observed in the response to ISO in the three groups of rabbits. Feeding of the normal rabbits appeared to produce a slight increase in the release of lactate by EPI which corresponds to a slightly higher level of glycogen in the skeletal muscle of the fed group [7]. Elimination of acute insulin release did not alter the elevations in lactate achieved with either ISO or EPI.

Effects of propranolol on EPI- and ISO-induced changes in plasma glucose, lactate and glycerol. As shown in Fig. 2, propranolol antagonized the EPI-induced rise in plasma lactate (100 per cent) more than the rise in plasma glycerol (70 per cent). In contrast, the increase in plasma glucose level produced by EPI was not changed significantly by blockade of beta-receptors with propranolol.

Figure 3 illustrates the effects of propranolol on changes in glucose, lactate and glycerol produced by ISO. As was the case with EPI, the lactate response was inhibited considerably (about 75 per cent) by pretreatment with propranolol. The plasma glucose response to ISO was inhibited about 50 per cent at 30 and 45 min by beta-receptor blockade. The increase in plasma glycerol caused by ISO was the least antagonized of the responses (about 33 per cent inhibition).

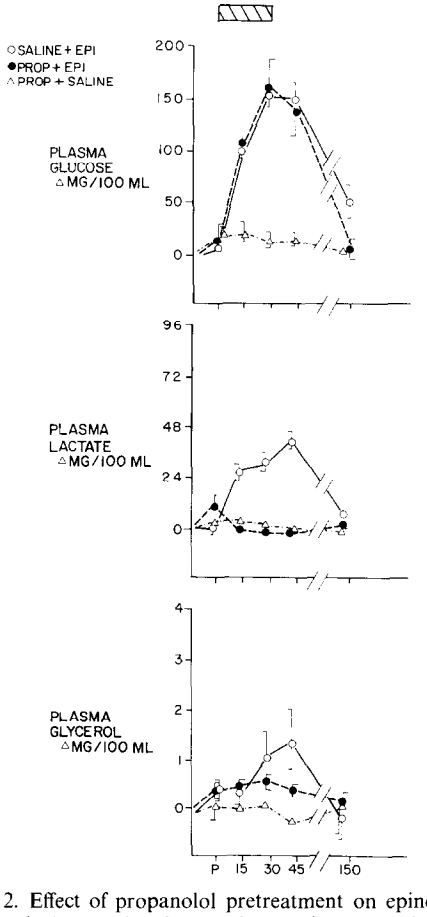

Fig. 2. Effect of propanolol pretreatment on epinephrine-induced changes in plasma glucose, lactate and glycerol levels in fed rabbits. Propranolol (P) (0.3 mg/kg/min) inhibited the increases in lactate and glycerol levels but did not antagonize the rise in plasma glucose. Each point represents the mean ± S. E. of determinations in each of five rabbits.

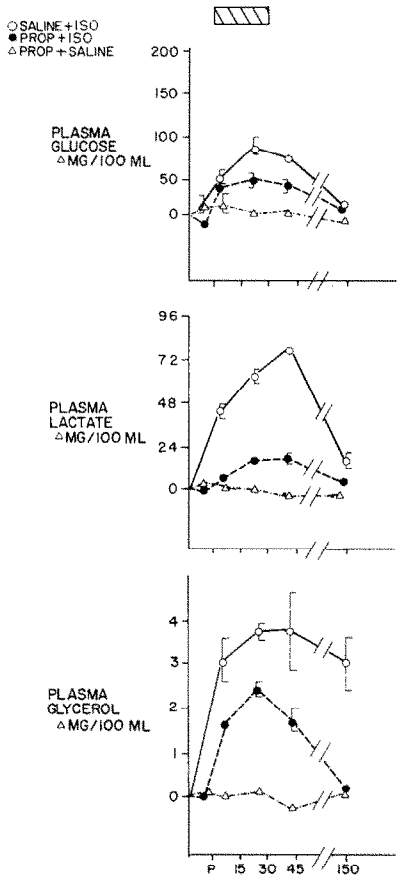

Fig. 3. Inhibitory effects of propanolol on isoproterenol-induced changes in plasma glucose, lactate and glycerol levels in fed rabbits. Propranolol (0.3 mg/kg/min) was infused over a 30-min period prior to the infusion with isoproterenol (30 μg/kg/min). The substrate level immediately after the infusion with propanolol is indicated by the letter "P." Propranolol inhibited changes in the following order: lactate > glucose > glycerol. Each point is the mean ± S.E. of no fewer than five determinations from each of five animals.

DISCUSSION

The results of these experiments in the intact rabbit appear to be relevant to the question of the relative importance of the direct effects of catecholamines on liver metabolism versus the indirect consequences of the extrahepatic actions of these agents [2, 8]. Equieffective hyperglycemic doses of EPI and ISO had different activities in raising blood levels of glycerol and lactate in normal fasted rabbits. EPI induced a rather small increase (27 mg/100 ml) in peripheral levels of lactate compared to the rise after administration of ISO (75 mg/100 ml). Similarly, glycerol levels were slightly elevated after infusing EPI (1.30 mg/100 ml), whereas ISO produced a marked rise (4.55 mg/100 ml). The limited release of lactate and glycerol produced by EPI suggests that these gluconeogenic substrates probably do not serve as the main source of glucose that is mobilized by this catecholamine. On the other hand, muscle and adipose tissue may be the primary target organs involved in the metabolic response to isoproterenol. The abrupt increase in glycerol and lactate after the i.v. infusion of ISO makes possible a greater rate of removal and utilization of both substrates for glucose production [9–13]. Results from earlier experiments involving the effects of propranolol on catecholamine-induced changes in blood glucose and tissue glycogen give further support to these interpretations [14].

Feeding did not change appreciably the effects of ISO on plasma glycerol, lactate or glucose levels; however, the release of all three substrates by EPI was affected to varying degrees. As a consequence of this change in nutritional state, the hyperglycemia produced by EPI was enhanced considerably. It is improbable that this augmented glucose response can be the consequence of an increase in gluconeogenesis because in the fed state the productive activity of gluconeogenic pathways should be minimal [15]. A more suitable explanation can be extracted from a previous paper from this laboratory wherein it was reported that liver glycogen content was three times greater in fed rabbits than in rabbits fasted for 24 hr [2]. Thus, it seems reasonable to speculate that liver glycogen stores constitute the primary source of glucose for the graded response to intravenously administered epinephrine in fed versus fasted rabbits.

Alloxan-diabetic, fed rabbits and normal, fed rabbits showed comparable increases in glycerol and lactate levels in response to ISO. Interestingly, the hyperglycemic response to ISO was potentiated in a striking manner in alloxan-treated rabbits. Observations from this laboratory have demonstrated that ISO promotes the acute release of insulin in the normal rabbit [7]; hence, it seems likely that a diminished release of insulin from the β-cell was the factor responsible for the enhanced hyperglycemia to ISO in diabetic rabbits. In alloxan-treated animals, the suppressant effect of acute insulin release on hepatic glycogenolysis and peripheral utilization is virtually eliminated; therefore, isoproterenol produces a greater hyperglycemic effect. An augmented hyperglycemic response has been demonstrated to dibutyryl cyclic AMP in diabetic dogs [16], and to glucagon in diabetic rabbits [17] and dogs [18].

The glycerol-releasing effect of ISO, unlike the hyperglycemic response, was not enhanced in rabbits deprived of normal pancreatic β-cell function. Even in normal rabbits, adipose tissue would be exposed to a much smaller proportion of the insulin released by ISO than would the liver. For this reason, it seems reasonable to assume that the direct lipolytic effect of ISO would overcome the peripheral effects of insulin released endogenously.

EPI generated similar elevations of blood glucose and lactate in both fed normal and fed diabetic rabbits. The lack of a change in the hyperglycemic response to EPI in alloxan-diabetic, insulin-treated rabbits compared with normal rabbits is compatible with the evidence that this catecholamine inhibits the release of insulin in normal rabbits [7]. The delayed increase in glycerol levels after an infusion with EPI in diabetic rabbits may have been the result of a diminished insulin response to EPI-induced hyperglycemia.

The marked suppression of catecholamine-induced increases in lactate levels by propranolol indicates the high sensitivity of muscle phosphorylase to beta

blockade regardless of whether the agonist is EPI or ISO. Adipose tissue reactivity to catecholamines, as measured by changes in glycerol levels, appears to be only moderately inhibited by propranolol. Our results are in general agreement with the data of Spitzer [19], although we were using fully nourished rabbits and she used fasted animals. Propranolol failed to attenuate the hyperglycemic response to epinephrine and only reduced the response to isoproterenol by about 50 per cent in fed rabbits. Earlier experiments using fasted animals showed that ISO-induced hyperglycemia could be virtually eliminated by pretreatment with propranolol whereas the response to epinephrine was not inhibited appreciably [1]. These data suggest that ISO-induced hyperglycemia in rabbits is more dependent upon mechanisms involving gluconeogenesis than is the hyperglycemic response to epinephrine.

It is often argued that the relative effectiveness of different catecholamines in raising blood glucose levels in the intact animal cannot be equated with their relative effectiveness in promoting hepatic glycogenolysis, because the hyperglycemic response is dependent upon the composite actions of catecholamines on a multiplicity of interrelated systems. However, our results with EPI in the presence of propranolol in the fed rabbit suggest that the capacity of EPI to raise blood glucose can be related to a primary effect on hepatic glycogenolysis because beta-receptor blockade eliminated much of the contribution by metabolic substrates from skeletal muscle and adipose tissue without altering the elevation of glucose levels.

In summary, the following points are noted: (1) alterations in the state of nourishment influenced EPI-induced changes in plasma glucose and lactate in normal rabbits whereas metabolic responses to ISO were not influenced appreciably; (2) suppression of insulin release by treatment with alloxan augmented markedly the hyperglycemic response to ISO in rabbits, but the response to EPI was not altered significantly; (3) pretreatment with propranolol inhibited catecholamine-induced increases in plasma lactate more than glycerol in normal fed rabbits; the hyperglycemic response to ISO was reduced about 50 per cent whereas the response to EPI was not inhibited; and (4) it is concluded that EPI-induced changes in plasma glucose levels in the rabbit are influenced to a greater degree by the state of nourishment than by the functional status of pancreatic β-cells. In contrast, ISO-induced hyperglycemia is influenced to a far greater extent by the capacity to release insulin than by the state of nourishment.

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