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EFFECT OF ISOPROTERENOL ON GLUCOSE TURNOVER AND INSULIN SECRETION IN THE NORMAL DOG*

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Abstract—Infusion of epinephrine in the dog causes a sustained hyperglycemia without increasing plasma insulin, whereas infusion of isoproterenol causes a transient hyperglycemia and increased plasma insulin. To investigate the basis for these differences, rates of hepatic glucose production and overall glucose uptake were determined using a primer-constant infusion of [3-^{3}H]glucose in normal dogs, and these were correlated with plasma insulin levels. Infusion of epinephrine (0.2 μg/kg/min) for 3 hr caused a prompt and persistent hyperglycemia. Concomitantly glucose production was increased but returned to normal by 90 min. Plasma insulin and glucose uptake did not increase. Isoproterenol infusion (0.2 μg/kg/min) for 3 hr caused a transient hyperglycemia. Glucose production and uptake were increased during the entire period. Plasma insulin was also elevated. The transient hyperglycemic response to isoproterenol, therefore, is not due to inadequate glucose production. Infusion of the beta-adrenergic blocker, propranolol, prevented the hyperglycemia and increased hepatic glucose production normally produced by isoproterenol.

Administration of epinephrine and isoproterenol causes a rise in the blood glucose concentration of a number of species (for reviews see Refs 1–3). The mechanism of the hyperglycemic effect of epinephrine has beeen thoroughly investigated and there has been general agreement that epinephrine causes a marked inhibition of glucose uptake [4]. Studies using isotope dilution techniques have revealed that continuous infusion of epinephrine also produces a prompt increase in hepatic glucose output which returns to control values in 1 hr, while hyperglycemia persists for at least 2 additional hr [4]. This transient response could not have been deduced from the observed plasma glucose concentrations.

Isoproterenol injection produces a modest hyperglycemic effect in several species such as rabbit [5–7], fasted rat [8–10] and dog [11, 12]. However, a lack of effect has been reported in man [13–16] an in the fed rat [10, 17, 18]. The deficient hyperglycemic response to isoproterenol remains to be explained, but it has been suggested that insulin secretion evoked by isoproterenol may blunt or prevent the expected increase in glucose production.

The present study uses an isotope dilution technique to explore the effect of isoproterenol on hepatic glucose output and overall glucose uptake in the normal dog. These measurements are correlated with plasma insulin concentrations.

MATERIALS AND METHODS

Experiments were carried out on normal, conscious, trained dogs, of either sex, at about 18 hr after the daily feeding. Serial blood samples were collected from the jugular vein via an indwelling polyethylene (P.E. 190) tubing inserted percutaneously shortly before the experiment. The tracer isotope and drugs were infused into the saphenous vein through a smaller polyethylene tubing (P.E. 50).

[3-^{3}H]glucose (glucose-3-T; New England Nuclear) was administered as a priming injection followed immediately by a constant infusion [19]. The ratio of priming injection to infusion was about 100 to 1. A 3-hr period from the start of the tracer infusion was allowed for determination of the control values for glucose production and uptake, and then the various individual drugs and combinations of drugs were administered.

Blood samples were collected in heparinized syringes and placed into chilled test tubes. After centrifugation, the plasma was separated and an aliquot was immediately deproteinized with $ZnSO_4$ and Ba $(OH)_2$ according to Somogyi [20]. An aliquot of each filtrate was analyzed for glucose by the glucose oxidase method [21] using a Beckman glucose analyzer. Another aliquot was used to determine the glucose specific activity [19]. Plasma free fatty acids were determined on samples of plasma by the method of Dole and Meinertz [22], and plasma insulin by the radioimmunoassay method of Hales and Randle [23] using a kit (Schwarz-Mann, Orangeburg, NY).

Rates of hepatic glucose production and overall glucose uptake by tissues were calculated for the steady state, as described before [24]. During periods of changing plasma glucose concentrations, 0.7 of the initial glucose pool size was used as the rapidly mixing compartment [25].

The drugs used were L-epinephrine bitartrate (Suprarenin, Winthrop), isoproterenol hydrochloride (Isuprel, Winthrop) and *d,l*-propranolol hydrochloride (kindly donated by Ayerst Laboratories, New York, NY).

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RESULTS

The effect of infusion of epinephrine (0.2 μg/kg/min) into six normal dogs on various parameters is shown in Fig. 1. Plasma glucose increased promptly and remained elevated for the entire period of epinephrine infusion. The development of hyperglycemia was associated with increased glucose output by the liver. This effect, however, was transient, with glucose production returning to normal by 90 min. Plasma free fatty acids (FFA) levels showed a prompt but transient increase. Plasma insulin levels failed to increase despite the presence of elevated glucose levels and, likewise, glucose uptake by tissues did not increase. Indeed there is a relative inhibition of glucose uptake as evident from the persistence of hyperglycemia (90–180 min) despite a normal rate of glucose production. When glucose uptake is calculated in relation to plasma glucose, it may be expressed as metabolic clearance of glucose (glucose uptake ÷ prevailing plasma glucose concentration × 100), and this is also markedly depressed. These data agree with findings reported earlier [4].

The effects produced by the infusion of isoproterenol are strikingly different from the effects of epinephrine and are shown in Fig. 2. Isoproterenol infusion (0.2 μg/kg/min) into six normal dogs produced a significant but transient hyperglycemia. The rise in plasma glucose was due to increased glucose production which persisted for the entire period of observation. This effect was not reflected in the plasma glucose values because glucose uptake was also elevated. Plasma insulin levels increased promptly and declined slowly toward control values. The persistence of increased glucose uptake beyond

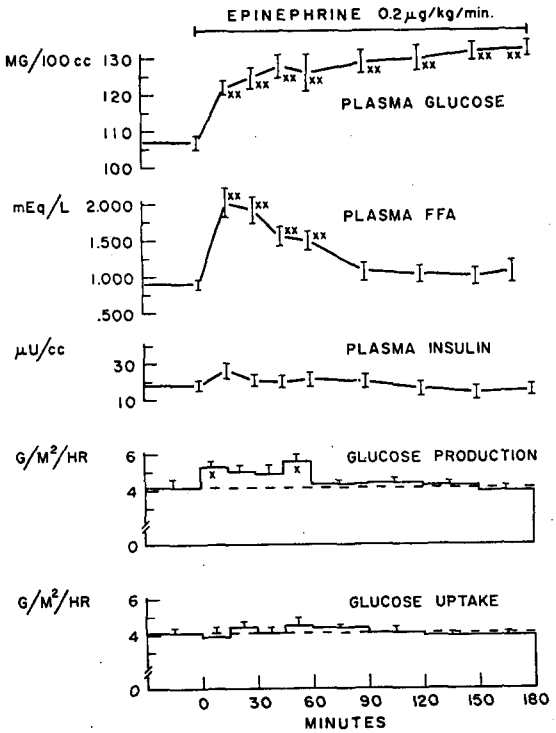

Fig. 1. Effects of infusion of epinephrine (0.2 μg/kg/min) in six normal dogs on plasma concentrations of glucose, FFA and insulin and on rates of glucose production and uptake. Statistical significance, based on Student's *t*-test, is indicated as follows: ,x = $P < 0.05$; xx = $P < 0.01$. Epinephrine caused significant increases in plasma glucose and FFA. Glucose production shows a transient increase.

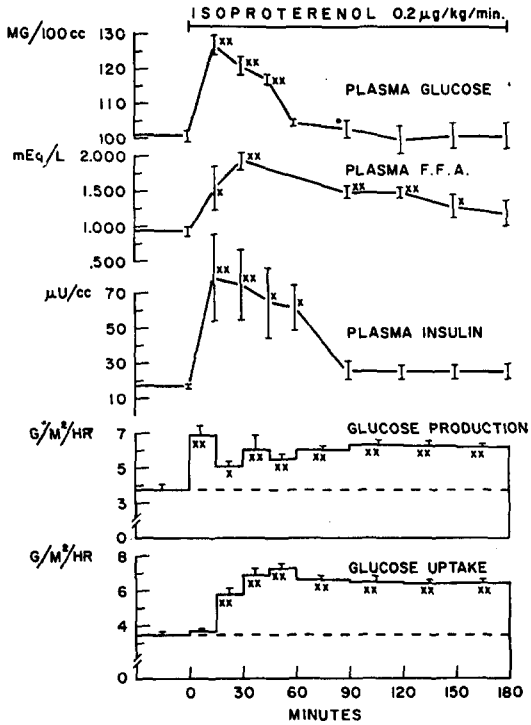

Fig. 2. Effects of infusion of isoproterenol (0.2 μg/kg/min) in six normal dogs. Statistical designations are as in Fig. 1. Isoproterenol caused only a transient increase in plasma glucose, but glucose production and uptake remained elevated during the entire period of infusion. Plasma insulin was also markedly elevated.

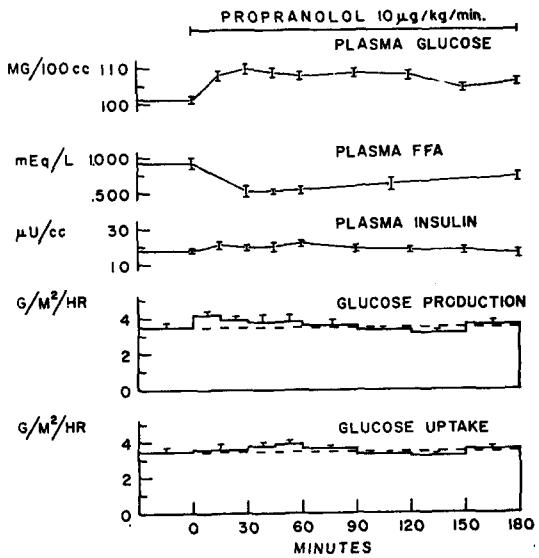

Fig. 3. Effects of infusion of propranolol (10 μg/kg/min) in four normal dogs. Propranolol caused small elevations in plasma glucose and small decreases in plasma FFA, but the changes were not statistically significant.

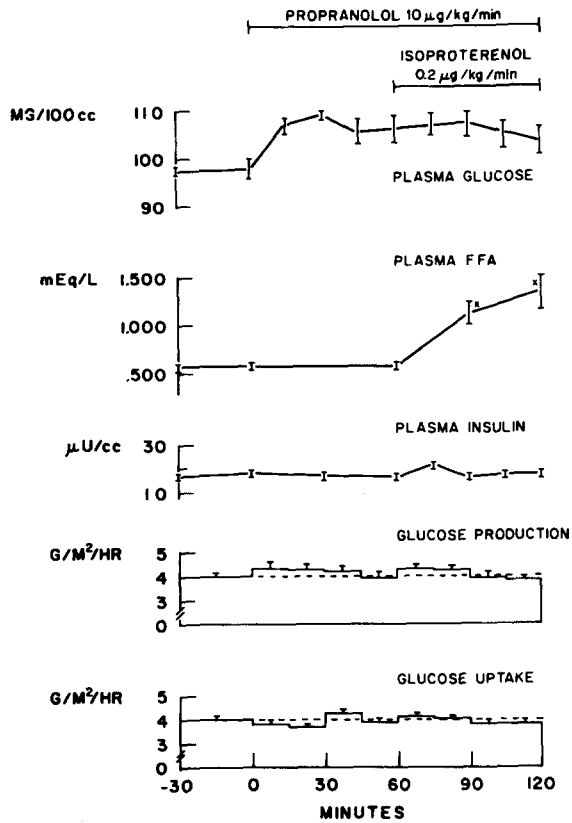

Fig. 4. Effects of combined propranolol and isoproterenol infusion in four normal dogs. Propranolol blocked the usual isoproterenol-induced increases in plasma glucose and insulin concentrations and in glucose production, but did not block the rise in plasma FFA ($x = P < 0.05$).

the time of return of insulin to control values probably reflects a residual effect of the preceding high levels of insulin. Plasma FFA concentration rose and remained significantly elevated for all but the last value at 180 min.

The effect of beta-blockade on the above responses to isoproterenol was evaluated using propranolol. As seen in Fig. 3, propranolol infusion alone (10 μg/kg/min) into four normal dogs produced insignificant changes on several parameters. The effects of propranolol on the responses to isoproterenol are shown in Fig. 4. Beta-blockade prevented the usual rise in plasma glucose and insulin and the increase in glucose production. Glucose uptake did not increase. Plasma FFA levels still increased in the presence of beta-blockade.

DISCUSSION

The present study reveals that epinephrine and isoproterenol differ markedly in their effects on glucose metabolism. Furthermore, these differences could not have been predicted from the observed blood glucose responses. In the case of epinephrine, the rise in plasma glucose concentration was sustained throughout a 3-hr period of infusion, and it might be reasonable to assume a continued glycogenolytic response and hence increased hepatic glucose output. However, measurement of hepatic glucose production revealed that epinephrine infusion caused a prompt but transient increase in glucose production which caused the initial rise in plasma glucose levels. The induced hyperglycemia was sustained by impairment of glucose uptake by tissues due either to the absence of the release of insulin normally evoked by hyperglycemia or due to a direct inhibitory effect of epinephrine [26–28].

The transient stimulation of hepatic glucose output remains to be explained, but liver glycogen deficiency can be ruled out. Such treated animals have ample liver glycogen [7] and are still capable of responding to other glycogenolytic agents such as glucagon (Altszuler, unpublished observations). It is also unlikely that the transient effect on hepatic glucose output is a secondary effect of epinephrine action at some other peripheral sites because it was also observed in the isolated perfused rat liver [29]. Such transiency was also reported during glucagon administration [30]. Transient responses may perhaps be more common for glycogenolytic agents administered in small doses but glucagon in larger doses (10 μg/kg/hr) will stimulate glycogenolysis for 6 or more hr until glycogen is depleted (Altszuler, unpublished observations). Furthermore, the hepatic response to isoproterenol, as shown in the present study, persists beyond the duration of the epinephrine effect on the liver, thus ruling out deficiency of glycogen mobilization as a limiting factor in the transient response to epinephrine.

Isoproterenol administration, in contrast to epinephrine, produced a prompt but transient hyperglycemia. Based on this response alone, the dog might appear to be unresponsive to the hyperglycemic effect of isoproterenol and resemble responses in man and the fed rat. However, there was a rise in plasma insulin levels, as was seen also in the rat [18, 31] and man [32]. It would seem reasonable to speculate that elevated plasma insulin levels would decrease glucose production and thereby account for the transient or insignificant effect of isoproterenol on plasma glucose [18]. However, the present measurements show a significant and sustained increase in hepatic glucose production which obviously overrides any inhibitory effect of insulin.

Isoproterenol differs from epinephrine in another significant way in that it does not produce the same degree of inhibition of glucose uptake. Some insight into the mechanism of this dibutyryl cyclic AMP inhibition is derived from experiments infusing dibutyryl cyclic AMP in normal dogs. Such infusion produced effects similar to those of isoproterenol on plasma insulin levels and glucose uptake [33]. However, when glucose uptake is presented as metabolic clearance, as discussed in Results, it is seen that epinephrine decreases metabolic clearance, while dibutyryl cyclic AMP and isoproterenol increase it. The increase in metabolic clearance is not as marked as that resulting from glucose infused at a rate to produce a similar hyperglycemia, thus suggesting that dibutyryl cyclic AMP and isoproterenol still exert a relative inhibition of glucose uptake.

The increased hepatic glucose production evoked by isoproterenol is a function of stimulation of the beta-adrenergic receptors, since it is blocked by propranolol. Alpha-receptor blockade using phentolamine does not prevent isoproterenol-induced hyper-

glycemia [34]. Increased insulin secretion during isoproterenol infusion is probably evoked in part by the induced hyperglycemia, since it tends to decline as the plasma glucose concentration returns to control values. Isoproterenol may also increase insulin secretion directly.

The insulin response observed here is in agreement with the concept that stimulation of alpha-receptors of the islet of Langerhans prevents their response to hyperglycemia whereas stimulation of beta-receptors does not. Additional insight regarding the inhibitory effect of epinephrine on insulin secretion may be gained from the findings with dibutyryl cyclic AMP administration. If the epinephrine inhibitory effect on insulin secretion were mediated via the formation of cyclic AMP, it might be expected that administration of dibutyryl cyclic AMP should mimic the effects of epinephrine. However, dibutyryl cyclic AMP infusion, while causing a hyperglycemia, does not prevent the expected stimulation of insulin secretion [33]. This suggests that the inhibitory effect of epinephrine resides at some point prior to the increased cyclic AMP formation, at the level of adenyl cyclase or perhaps other membrane-related structures.

The effect of isoproterenol to increase plasma FFA is in agreement with reports by others [13, 16, 35]. Propranolol also has been reported to block this effect of isoproterenol when the latter was given in small doses [35]. Larger doses of isoproterenol could surmount the blocking effect of propranolol. The significance of these interactions at excessively large doses of agonist and antagonist is uncertain. The present data indicate only that it is possible to select a dose of propranolol which blocks the increased glucose production of isoproterenol effectively without altering the effect on FFA. The relevance of this dissociation of blocking effects of propranolol remains to be explained.

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