Effects of Different Prostaglandins on Glucose Kinetics in the Rat

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SUMMARY

The primed constant infusion of glucose-2-^{3}H was used to study the influence of prostaglandins (PGs) infusion (2 μg./min.) on glucose turnover in intact rats in the postabsorptive state. While PGA_1 did not exert any appreciable effect on glucose production by the liver and on plasma glucose levels, prostaglandins of E-series significantly increased hepatic glucose output and consequently plasma glucose levels. In this regard, PGE_1 appeared more effective than PGE_2. In some experiments PGE_1 was infused for two hours at the dose of 1.5 μg./min.: glucose concentration rose to a maximum of 127 per cent above the control values and glucose production reached during the second hour of the infusion a level twice the base line (from 8.1±0.5 to 16.9±0.5 mg./kg. body weight/min.). These changes were paralleled by a proportional increase in glucose uptake by peripheral tissues which reached a level very near to that of hepatic output toward the end of the infusion.
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Numerous investigations have firmly established that prostaglandins (PGs) may influence many aspects of carbohydrate metabolism. The experiments performed in vivo have not yielded unequivocal results because of several factors, e.g. type of prostaglandin, doses employed, animal species. However, a clear hyperglycemic action has been reported in many species including dogs,[1-3] rabbits,[4,5] mice,[6] rats and guinea pigs.[7] This effect was initially ascribed to a reflex release of adrenal catecholamines secondary to the lowered blood pressure rather than to a primary action of PGs, and this conclusion was based on the lack of hyperglycemic response in adrenal demedullated animals.[5,7,8] On the other hand, more recent studies demonstrated that PGs may directly affect some aspects of carbohydrate metabolism in various experimental conditions. In this regard, PGE_1 was reported to inhibit glucose utilization by the isolated perfused rat liver[9] and to produce a significant decrease in glycogen synthetase activity not influenced by adrenal glands, beta-adrenergic and ganglionic block.[10] Moreover, numerous experiments performed in vitro on different tissues suggest that PGs possess an insulin-like activity in that they stimulate glucose uptake, glucose oxidation and triglyceride synthesis.[11-16] The present work was undertaken in order to further investigate the mechanism of PGs interactions with glucose metabolism "in vivo." For this purpose, experiments were designed to study the influence of different PGs on glucose kinetics by using the primed constant infusion of glucose-2-^{3}H. This technic was employed because it allows, with sufficient approximation to quantitate the changes in hepatic glucose output, an over-all uptake even though these rates are disparate and not stationary.

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MATERIALS AND METHODS

Experimental procedures. The experiments were conducted in male albino rats weighing between 325 and 420 gm. who were maintained on commercial pellet diet. After overnight fasting (eighteen to twenty hours), the animals were anesthetized with sodium thiopental (45 mg./kg. intraperitoneally) and the trachea was cannulated. A heating lamp was used to insure normal body temperature. Fifteen minutes after anesthesia was induced, 6 μc. of radioglucose (D-glucose-2-^{3}H, Amersham, S.A. 500 mCi/mmole) were administered intravenously as a priming dose, followed immediately by a constant infusion which was continued through the entire experimental period. The rate of infusion was 1.66 per cent of the priming dose per minute. Blood samples were withdrawn at 90, 105 and 120 minutes after the priming dose in order to calculate the base line values of glucose turnover rate. The infusion of PGs was started at 120 minutes and continued for forty-five minutes. Blood samples were collected every fifteen minutes during the infusion period and for thirty minutes thereafter. In some experiments the test infusion lasted 120 minutes; in this case sampling was done at thirty-minute intervals during the second hour of the test infusion. The effects of prostaglandin E_1, E_2 and A_1 were tested in twenty untreated rats subdivided into three groups. Crystalline preparations of PGs

(Upjohn Co., Kalamazoo, Mich.) were dissolved in 95 per cent ethanol and diluted with saline immediately before administration by constant infusion through a tail vein at the rate of 2 μg./min. For all the prostaglandins tested a total amount of about 0.03 ml. of ethanol was injected throughout the experiment. In another group of five rats, PGE_1 was infused at the rate of 1.5 μg./min. for two hours.

Analytical procedures. Blood samples were collected into heparinized tubes through a catheter placed in the jugular vein. In order to determine the specific activity of the plasma glucose, 50 μl. of plasma for each sample were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and the precipitate was centrifuged. An aliquot of the supernatant was used for glucose determination by the glucose oxidase method (Boehringer, Mannheim GmbH). Another aliquot was evaporated to dryness under vacuum at 70° C. to remove the ^3H_2O content of the plasma. This simple procedure completely isolates glucose-2-^3H, since all nonglucose radioactivity is present as ^3H_2O (vide infra). The dry residue was dissolved in 1 ml. of water and mixed with 9 ml. of liquid scintillator (Insta-Gel, Packard Instrument Co.). Radioactivity assay was done with a Nuclear-Chicago liquid scintillator spectrometer, and glucose specific activity was expressed as mμCi./mg.

Calculations. In the basal state, since no appreciable fluctuations in plasma glucose concentrations and specific activity occurred, the glucose turnover (mg./kg. body weight/min.) was calculated by the general equation applicable to all steady state systems: Ra = r/SA, where r is the infusion rate of the tracer (mμCi./kg./min.), and SA is the plasma specific activity of glucose at equilibrium (mμCi/mg.). Obviously, under these circumstances hepatic glucose output (rate of appearance, R_a) equals peripheral glucose uptake (rate of disappearance, R_d). In nonsteady states, it is possible to approximate satisfactorily these rates by using Steele's formulae (eq. 4a and 5a),[17] which give the averaged values of R_a and R_d for the interval between two consecutive samplings as:

$$Ra = \frac{r - [(P_1+P_2)/2]\,[(SA_2-SA_1)/(t_2-t_1)]}{(SA_1+SA_2)/2};$$

$$Rd = Ra - (P_2-P_1)/(t_2-t_1)$$

where r and SA are as above; P = pool size of glucose in milligrams per kilogram body weight; t_1 and t_2 = time in minutes of two consecutive samplings. In using Steele's equations the following assumptions were made: (1) The whole pool of glucose was introduced in the above formulae, since radioglucose mixes very rapidly in the rat glucose pool as reported by Shipley et al. for glucose-^{14}C (U)[18] and by Oldendorf and Kitano for similar-sized molecules.[19] We assumed, therefore, that "new glucose" released by the liver equilibrates almost immediately with the entire glucose pool. (2) The volume of distribution of glucose was assumed to be 25 ml./100 gm. of rat according to the finding of Shipley et al.[18] (3) Steele's method can be correctly applied to a single, rapidly reacting pool without recycling of tracer. In our experiments we used glucose-2-^3H because most of the tritium is irreversibly lost as water from position 2 during the isomerization of the hexose-6-phosphates and less than 5 per cent is incorporated into glycogen and other compounds.[20,21] When glucose-^{14}C(U) is used as tracer, a rapid recycling of radiocarbon from nonglucose compounds takes place, and this in turn leads to an underestimation of the glucose turnover.[20-22] This source of error, as outlined by Shipley and Clark, is particularly important in the rat, since in this animal radiocarbon is incorporated very rapidly in nonglucose compounds.[23]

All values are presented as mean ± S.E. and statistical analysis was performed by standard technics.[24]

RESULTS

Table 1 summarizes the effects of prostaglandins' infusion (2 μg./min.) into intact rats. PGE_1 infusion resulted in a marked increase in plasma glucose which remained at significantly higher levels also in the postinfusion period. Hyperglycemia was invariably associated with a highly significant increment in hepatic glucose output throughout the experimental period. An essentially similar response was recorded in rats receiving PGE_2 even if the changes in plasma glucose and hepatic output were of minor extent in comparison to those brought about by PGE_1. By contrast, PGA_1 was unable to produce the metabolic changes observed with E-series PGs and rather a slight tendency to hypoglycemia took place during the infusion. The response to the long-standing infusion of PGE_1 (1.5 μg./min.) is illustrated in figure 1. In these experiments PGE_1 exerted a spectacular hyperglycemic effect with a maximum glucose concentration of 127 per cent above the control value at the end of the infusion. Hepatic glucose production progressively increased during the first hour, and thereafter it stabilized at a level twice the base line value during the second hour at which time a new steady state was reached. It is noteworthy that glucose uptake by peripheral tissues rose immediately after the beginning of the infusion and reached a level very near to that of hepatic output toward the end of the infusion. This would clearly indicate that hyperglycemia is

TABLE 1

Effects of infusion of different prostaglandins (2 μg./min.) on plasma glucose levels and hepatic glucose production in normal rats.

		min. after start of PGs infusion					
		0	0-15	15-30	30-45	45-60	60-75
Plasma Glucose (mg./100 ml.)	PGE_1 (9)	89±4	109±6†	121±5‡	137±6‡	137±7‡	134±6‡
	PGE_2 (6)	80±7	90±12*	99±14*	102±13*	96±7†	95±6*
	PGA_1 (5)	87±5	89±7	79±6*	83±6	86±7	91±7
Glucose Production (mg./kg./min.)	PGE_1 (9)	6.7±0.4	11.5±0.8‡	11.0±0.7‡	12.3±0.8‡	11.6±0.8‡	10.5±0.6‡
	PGE_2 (6)	7.2±0.7	10.2±1.8*	10.4±1.8*	10.2±1.3*	10.0±1.0*	9.6±0.6*
	PGA_1 (5)	7.6±0.5	8.8±0.7	7.6±0.3	7.8±0.3	8.6±0.6	8.9±0.7

* $p<0.05$ † $p<0.01$ ‡ $p<0.001$
Number of rats in parentheses.

promoted by the increased release of glucose by the liver. This point of view is also corroborated by the behavior of the specific activity of glucose which rapidly declined soon after the beginning of the infusion.

DISCUSSION

In the basal state the turnover rate of glucose, expressed as the average of all experiments, was 7.3 mg./kg. body weight per minute. This value, which represents a "true turnover rate" because of the lack of radiocarbon recycling, is in good agreement with previous findings. In fact, in many tracer studies performed in the rat with glucose-^{14}C(U), the mean values reported range from 5.5 to 6.2 mg./kg. body weight/min.[18,25,26] On the other hand, in studies with simultaneous injections of glucose-2-^{3}H and glucose-^{14}C(U), the fractional turnover rate of glucose was 1.5 times higher with tritiated glucose as compared to glucose-^{14}C(U).[20]

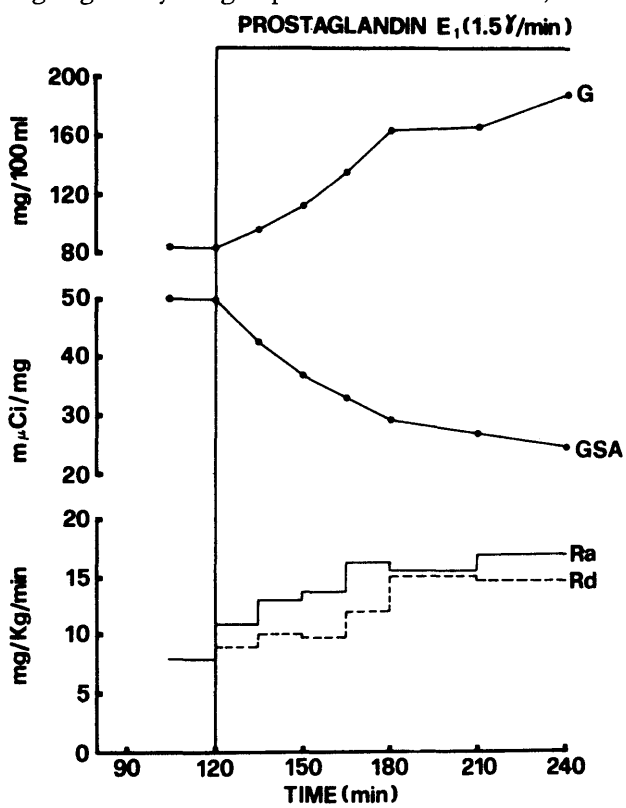

FIG. 1. Changes in plasma glucose (G), specific activity of glucose (GSA), hepatic glucose production (Ra) and over-all tissue uptake (Rd) in normal rats during a two-hour prostaglandin E_1 infusion at the rate of 1.5 μg./min. All points are means of data from five animals.

The turnover rate of glucose was measured in our experiments before and during the infusion of several PGs, since no attempt as yet has been made to quantitate in kinetic terms the well recognized hyperglycemic action of PGs. It is clearly apparent from analysis of the results that PGE_2 and even more PGE_1 exert a stimulatory effect on hepatic glucose production and consequently an enhancement of plasma glucose levels occurs. Since the experiments were carried out in the postabsorptive state, an increased hepatic glycogenolysis is certainly responsible for much of the increase in glucose production by the liver. It is likely that some gluconeogenic pathways are also activated by PGE_1 because of the marked and persistent hyperglycemia observed in the experiments as illustrated in figure 1.

Unlike E-series PGs, PGA_1 appears to be completely ineffective on glucose production but a slight tendency to hypoglycemia was observed. This different response yields further evidence of the variable effects of PGs due to the animal species under study. In fact, we found in previous works performed in dogs that PGA_1 produced hyperglycemia and glucose intolerance after intravenous glucose loading[3]; on the other hand PGA_1 has been reported to not modify blood glucose levels in man.[27]

The most notable result was observed in rats receiving PGE_1 for two hours in which glucose uptake by

tissues significantly increased over the control values and during the second hour of infusion it was almost doubled. Therefore, PGE_1-induced hyperglycemia appears to be somewhat different in nature from that brought about by epinephrine which stimulates hepatic glucose release but concomitantly inhibits glucose uptake by peripheral tissues.[28,29]

In conclusion, our studies do not permit us to hypothesize about the mechanism by which PGs enhance glucose turnover in the rat. However, a more complex mechanism than a catecholamine discharge secondary to the lowered arterial pressure seems to underlie the observed changes in glucose turnover; therefore, further investigations are needed in this area.

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