Life Sciences, Vol. 34, pp. 1699-1706
Printed in the U.S.A.

Pergamon Press

EFFECTS OF LIPOXYGENASE AND CYCLOOXYGENASE INHIBITORS ON GLUCOSE-STIMULATED INSULIN SECRETION FROM THE ISOLATED PERFUSED RAT PANCREAS

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(Received in final form February 26, 1984)

Summary

Some of the metabolites of arachidonic acid formed in the lipoxygenase and cyclooxygenase pathways stimulate insulin release. We studied the relative importance of each of these pathways in the modulation of glucose-induced insulin release by using inhibitors of arachidonate metabolism. Perfusion of the isolated rat pancreas with two chemically different inhibitors of cyclooxygenase, flurbiprofen and sodium salicylate, markedly inhibited prostaglandin E_2 release, but had little effect on glucose-induced insulin release or on potentiation of insulin release caused by prior exposure to glucose. On the other hand, nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor, not only inhibited both phases of glucose-induced insulin release but also abolished the potentiation effect. These effects of NDGA prevailed, when it was administered together with flurbiprofen, which caused profound inhibition of prostaglandin E_2 release. We conclude that 1) lipoxygenase pathways play a dominant role in glucose-stimulated insulin release, and 2) endogenous lipoxygenase metabolites influence the potentiating effect of glucose on the release of insulin in response to a subsequent stimulation.

We have shown that the administration of exogenous prostaglandins (PGs) induces insulin release from the isolated perfused rat pancreas when the glucose concentration in the perfusion medium is physiological (1-3). Using perifused islets, also Burr and Sharp observed a stimulation of insulin release by PGE at a low glucose concentration (4). Other investigators failed to observe an effect of PGs under these conditions (5,6). Conflicts also arose when glucose-stimulated insulin secretion was studied; some authors reported enhancement (5,6), while others suppression of insulin release (4,7-9).

Experiments with cyclooxygenase inhibitors also yielded disparate results, suggesting inhibition, stimulation or lack of an effect by endogenous PGs on insulin release (7,10-13). Islets incorporate radiolabeled arachidonate into various phospholipids (14,15). Phospholipases liberate arachidonate from the phospholipids; these enzymes are activated by calcium and glucose (16). In islets, arachidonate is then converted by cyclooxygenases to PGs (14,15). Intact or sonicated rat pancreatic islets, and neonatal rat pancreatic cells in culture release a variety of PGs (8,14,17,18). Recently, attention has shifted to products of the lipoxygenase pathway of arachidonate metabolism as possible mediators of insulin secretion (19-21); the islets have been shown to produce these metabolites as well (16,20,22).

0024-3205/84 $3.00 + .00

Our aim in this investigation was to study the role of endogenously generated metabolites of arachidonate in glucose-stimulated insulin secretion by the isolated perfused pancreas. We studied the effects of a lipoxygenase inhibitor, nordihydroguaiaretic acid, two cyclooxygenase inhibitors, flurbiprofen and sodium salicylate, and the combination of flurbiprofen and nordihydroguaiaretic acid on the dynamics of glucose-induced insulin and PG release. We also explored the influence of these compounds on the "priming" effect of antecedent administration of glucose on subsequent glucose-induced insulin release (23,24). We found that the inhibition of lipoxygenases not only diminished glucose-induced insulin release, but also nearly abolished the priming or potentiating effect of a previous glucose stimulus.

Materials and Methods

Young, male Sprague-Dawley rats weighing 250-300 grams (Charles River Laboratories, Wilmington, MA) were fasted overnight. The pancreas of an animal was surgically removed, affixed to a fiberglass platform in anatomically correct position, and transferred to a multiple-channel perfusion apparatus (2). Perfusion buffer entered the aortic cannula at a rate of 2.5 ml/min and was collected from a portal vein cannula. Exocrine secretion was drained via a duodenal tube. The perfusion buffer solution, which was continually gassed with 95% oxygen and 5% carbon dioxide to maintain pH at 7.4 was composed of 118.5 mM sodium chloride, 3.5 mM potassium chloride, 1.0 mM magnesium sulfate, 1.2 mM monobasic potassium phosphate, 2.5 mM calcium chloride, 24.9 mM sodium bicarbonate, 40 g/liter dextran and 2 g/liter bovine serum albumin. The basal perfusion solution also contained 5.6 mM D-glucose.

The pancreas was equilibrated with the basal perfusion solution for 30 min. Fifteen minutes after the start of the experiment, the channel was switched to one that contained a perfusion solution with 16.7mM glucose. This stimulatory period lasted 15 min (Period 1, 15-30 min) and was followed by a 30-min perfusion with the basal solution and a second 15-min period of perfusion with 16.7mM glucose (Period 2, 60-75 min). In the experiments where the inhibitor drugs were employed, the perfusion of nordihydroguaiaretic acid (NDGA, 10μM, Sigma), flurbiprofen (FLR, 1μM, Upjohn), or sodium salicylate (SAL, 1.2mM, Sigma) was begun at minute 5 and lasted throughout the rest of the experimental period. Effluent, collected every minute with the aid of a fraction collector, was quickly chilled, then frozen. Selected samples were radioimmunoassayed for insulin (25), and PGE_2 (26).

The release of insulin and PGE_2 was quantified as areas under the curve (2). Incremental areas were obtained by subtracting baseline release; secretion rates corresponding to respective response areas were derived by multiplying the incremental areas by 2.5. The presented values are means $\pm$ standard errors. Statistics were calculated using a two-tailed Student's t-test and analysis of variance (27).

Results

Control Experiments

In the control experiments conducted in the absence of any drugs, insulin release occurred in a biphasic pattern during both periods of perfusion with 16.7mM glucose (Fig 1). Cumulative secretion of insulin during Period 2 of exposure to high concentration of glucose was greater ($p < 0.01$) than that observed during Period 1 (Table I).

PGE_2 was released in a slowly cumulative and steady manner; an effect of changes in glucose concentration was not discernible (Fig 2). The incremental

amount of PGE_2 released during the period 5-90 min was 447±36 nmol. In a separate set of experiments, PGE_2 release during continuous perfusion with 5.6mM glucose was similar in magnitude and pattern (data not shown).

Effect of nordihydroguaiaretic acid

Starting at time 5 min, 10μM NDGA was perfused in order to inhibit pancreatic lipoxygenases. As compared to the control experiments, with NDGA, the late phase of glucose-induced insulin release was inhibited in Period 1, and early as well as the late phases of release were inhibited in Period 2 (Fig 1, Table I). The inhibitory effect of NDGA on glucose-induced insulin release was greater in Period 2 than in Period 1, so that the magnitudes of hormone release during Period 1 and Period 2 were no longer significantly different.

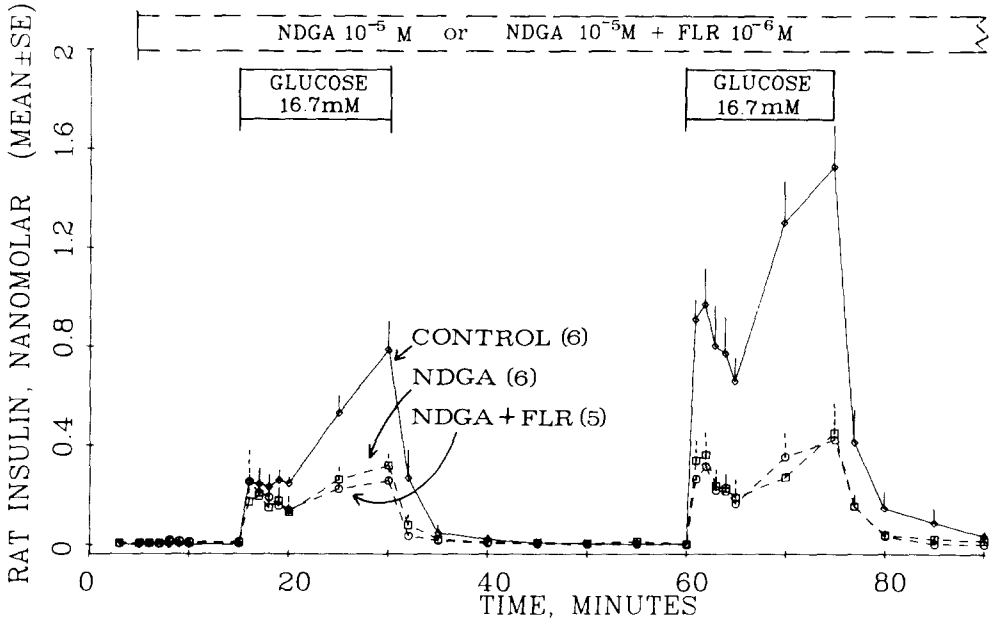

FIG. 1

Effect of nordihydroguaiaretic acid (NDGA) in the presence or absence of flurbiprofen (FLR) on glucose-induced insulin release during two 15-min periods. Numbers of experiments appear in parentheses.

At the concentration employed, NDGA also inhibited the release of PGE_2 to 44% of the amount released during the control experiments (Fig 2). The amount released from 5 to 90 min was 198±52 nmol ($p < 0.005$ vs control).

Effects of flurbiprofen and sodium salicylate

Since, with NDGA, the cyclooxygenase enzyme also appeared to have been inhibited, the effects of two chemically unrelated cyclooxygenase inhibitors were explored. In two separate series of experiments, from time 5 min onwards, the perfusion solution contained either 1μM FLR or 1.2mM SAL. Total PGE_2

release decreased to 13% of control with FLR, and to less than 1% with SAL (58±56 and -15±28 nmol, respectively, $p < 0.005$ vs control; Fig 2).

During Periods 1 and 2, both phases of glucose-induced release increased modestly, both with FLR and SAL (Table I). With FLR, insulin release was 124% of control during Period 1, and 130% during Period 2; with SAL the corresponding values were 133% and 148%. None of these increases were significant. With either drug, glucose-induced insulin release during Period 2 was greater ($p < 0.01$) than that during Period 1, with a ratio similar to that seen in control experiments.

TABLE I

Incremental Amounts of Immunoreactive Insulin Released by the Isolated Rat Pancreas

		Insulin Release						
		Period 1[a]			Period 2[a]			
	N	Acute (15-20min)	Delayed (20-35min)	Total (15-35min)	Acute (60-65min)	Delayed (64-80min)	Total (60-80min)	Ratio P2/P1
Control	6	2.7±0.4	16.8±2.4	19.5±2.7	9.4±1.4	36.8±4.2	46.3±2.2	2.4
NDGA 10μM	6	1.9±0.6	7.4±1.8[c]	9.2±2.3[b]	3.1±0.8[d]	10.4±2.5[d]	13.4±3.3[d]	1.5
FLR 1μM	6	4.1±0.9	20.1±3.7	24.1±4.5	13.5±2.0	46.8±4.5	60.3±6.4	2.5
SAL 1.2mM	6	4.2±1.3	21.7±6.2	25.9±7.5	17.4±4.7	51.2±12.1	68.6±16.7	2.6
NDGA 10μM + FLR 1μM	5	2.1±0.8	5.9±1.6[d]	8.0±2.3[c]	2.7±0.5[d]	10.0±2.7[d]	12.6±3.2[d]	1.6

Footnotes:
NDGA = nordihydroguaiaretic acid, FLR = flurbiprofen, SAL = sodium salicylate, administered from 5-min time point onwards. Glucose concentration was increased from 5.6mM to 16.7mM twice, from 15 to 30 min, and from 60 to 75 min.

[a] Values represent the means ± SE in nanomoles for the time periods indicated.

[b] $p < 0.05$; [c] $p < 0.01$, [d] $p < 0.005$ vs control.

Effect of nordihydroguaiaretic acid plus flurbiprofen

Because the selective inhibition of cyclooxygenase modestly promoted glucose-induced insulin release, in a final series of experiments 10μM NDGA was administered together with 1μM flurbiprofen to find out whether NDGA could exert its inhibitory effects at a time cyclooxygenase was profoundly inhibited. In these experiments, total PGE_2 release was inhibited to 9% of the corresponding levels observed during control experiments (Fig 2, 41±51 nmol, $p < 0.005$). This degree of inhibition was substantially less than the inhibition seen with NDGA alone (44% of control).

Combined administration of NDGA and FLR affected glucose-induced insulin release in a manner very similar to that seen with NDGA alone. During Period 1, the late phase, and during Period 2 both phases of insulin release were inhibited, and the difference in the magnitudes of the release during Periods 1

and 2 was abolished (Fig 1, Table I).

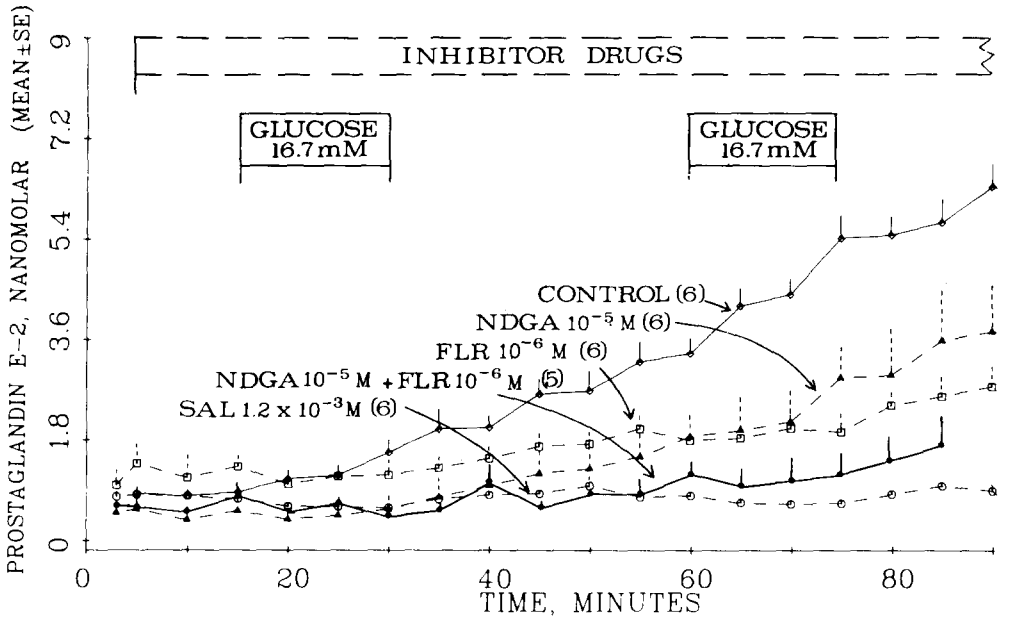

FIG. 2

Effect of nordihydroguaiaretic acid (NDGA), flurbiprofen (FLR), sodium salicylate (SAL) and NDGA + FLR on PGE_2 release from the isolated rat pancreas. Numbers of experiments appear in parentheses.

Discussion

The results of our study indicate that the lipoxygenase pathway of arachidonic acid metabolism plays a far greater role than the cyclooxygenase pathway in the release of insulin in response to glucose.

In this study we were able to monitor the activity of the cyclooxygenase but not the lipoxygenase pathway. We assume that with NDGA pancreatic biosynthesis of autacoids in the lipoxygenase pathways of arachidonate metabolism was inhibited. This effect of NDGA was documented conclusively in other systems (28). The assumption that inhibition of pancreatic lipoxygenases is the basis for the inhibitory action of NDGA on glucose-induced insulin release is plausible also for the following reasons: 1) lipoxygenase activity has been documented in pancreatic islets (16,22); 2) we have demonstrated that the administration of leukotriene B_4 (LTB_4), LTC_4, LTD_4 and LTE_4, all metabolites of arachidonate activated by 5-lipoxygenase, stimulate insulin release (29); and 3) Metz et al have shown that 12-hydroperoxyeicosatetraenoic acid, a 12-lipoxygenase-activated metabolite of arachidonate, stimulates insulin release (20).

At concentrations higher than those we have employed, NDGA has been shown to inhibit not only lipoxygenases, but also the cyclooxygenase (30). In the present study, the observed decreases in PGE_2 release indicate that,

unexpectedly, 10μM NDGA moderately inhibited the cyclooxygenase. We are confident that the inhibition of cyclooxygenase did not play any role in the inhibition of glucose-induced insulin release by NDGA, because 1) profound inhibition of PGE_2 release by two chemically unrelated inhibitors of cyclooxygenase (31,32) did not affect insulin release, and 2) the magnitude of inhibition of glucose-induced insulin release by NDGA was the same in the presence or absence of FLR.

Unlike our observation in the present experiments, we demonstrated previously that increases in the concentration of glucose in the range of 4-20mM induced dose-related increases in PG release from isolated rat islets incubated for 20 hours (33). We interpreted those findings as an indication of activation of phospholipase A_2 by glucose. Our failure to see an augmentation of PGE_2 release in conjunction with the increases in the concentration of glucose in the present study may be due to the relatively short duration of the experiments or to potential differences between glucose sensitivity of phospholipases in the exocrine and the endocrine cells of the pancreas.

In our control experiments, we observed the familiar phenomenon of potentiation of glucose-induced insulin release by antecedent administration of glucose. Inhibition of lipoxygenase with NDGA abolished this priming effect. The abolition of the priming effect could be due to a longer duration of exposure to NDGA in Period 2 than in Period 1 of glucose-induced insulin release. Nevertheless, our observation suggests that lipoxygenase-activated products of arachidonate metabolism participate in the priming action of glucose on glucose-induced insulin release. Prior studies assign an important role to the metabolism of glucose in the priming effect (23,24). We speculate that lipoxygenase products may contribute to the priming effect by enhancing the metabolism of glucose. The same mechanism could also explain the inhibition of glucose-induced insulin release which we observed with NDGA independent of the priming effect (Period 1). Validation of this proposal must await studies on the involvement of lipoxygenase products in the metabolism of glucose.

By using the perfused pancreas model, we were able to evaluate the influence of lipoxygenase products on different phases of insulin release. Both the early and the late phases of glucose-induced insulin release were inhibited in Period 2, but only the late phase was inhibited in Period 1. If one were to assume that the inhibition of the early phase release in Period 2 is related to the abolition of the priming effect, then a selective stimulatory effect of endogenous lipoxygenase products on the late phase of insulin release may be postulated.

In the present study we have not observed an effect of cyclooxygenase inhibitors on glucose-induced insulin release. In a previous study with the isolated perfused rat pancreas, under somewhat different experimental conditions, we observed that 1μM, but not 10nM flurbiprofen inhibited insulin release in response to 30-minute perfusions with 16.7mM glucose (34). A reason for this inconsistency is not readily apparent at this time. The magnitude of the problem of the inconsistency in the effects of cyclooxygenase inhibitors on insulin release is revealed by comprehensive review of the work of various investigators (35). Previously we provided ample evidence that certain prostaglandins stimulate insulin release (1-3). We believe that when arachidonate is abundant, such as when phospholipase A_2 is stimulated by glucose (16), variable degrees of inhibition of cyclooxygenase causes a diversion of proportionate amounts of arachidonate to the lipoxygenase pathway (36,37). The resultant increase in the biosynthesis of leukotrienes and hydroperoxy-fatty acids could then stimulate insulin release, thus masking the reduction in the stimulatory effect of prostaglandins. This hypothesis needs

to be tested further. Our present results with the cyclooxygenase inhibitors clearly document that the lipoxygenase pathways of arachidonate metabolism dominate in influencing insulin release.

We conclude that endogenous products of the lipoxygenase pathways of arachidonate metabolism amplify stimulated insulin release and participate in the potentiation of glucose-induced insulin release caused by antecedent administration of glucose.

Acknowledgements

Flurbiprofen and the reagents for the radioimmunoassay of PGE_2 were generous gifts of the Upjohn Company, Kalamazoo, Michigan. This work was supported in part by USPHS grants AM 21192, AM 07245, and AM 20572 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases.

References

1. S. PEK, T.-Y. TAI, A. ELSTER and S.S. FAJANS, Prostaglandins 10 493-502 (1975).
2. S. PEK, T.-Y. TAI and A. ELSTER, Diabetes 27 801-809 (1978).
3. J.O. AKPAN, M.C. HURLEY, S. PEK and W.E.M. LANDS, Can. J. Biochem. 57 540-547 (1979).
4. I.M. BURR and R. SHARP, Endocrinology 94 835-839 (1974).
5. D.G. JOHNSON, W.Y. FUJIMOTO and R.H. WILLIAMS, Diabetes 22 658-663 (1973).
6. S. NISHI, Y. SEINO, S. SEINO, K. TSUDA, J. TAKEMURA, H. ISHIDA and H. IMURA, Prostaglandins Leukotrienes Med 8 593-597 (1982).
7. J.R. McRAE, M. CHEN and R.P. ROBERTSON, Adv. Prostaglandin Thromboxane Res. 8 1287-1289 (1980).
8. S.A. METZ, R.P. ROBERTSON and W.Y. FUJIMOTO, Diabetes 30 551-557 (1981).
9. D. GIUGLIANO and R. TORELLA, Prostaglandins Med. 1 165-166 (1978).
10. J. GARCIA, M. ARATA, M.E. FERNANDEZ, E. ASTOLFI and J.C. BASABE, Horm. Metab. Res. 14 553-554 (1982).
11. S.H. BARON, J.C. DUNBAR and P.P. FOA, Horm. Metab. Res.14 437-438 (1982).
12. D. GIUGLIANO, A.S. LUYCKX and P.J. LEFEBVRE, Diabet. Metab. 6 39-46 (1980).
13. H. VIK-MO, K. HOVE and O.D. MYØS, Acta Physiol. Scand. 103 113-119 (1978).
14. M.H. EVANS, C.S. PACE and R.S. CLEMENTS, Jr., Diabetes 32 509-575 (1983).
15. S.G. LAYCHOCK, Diabetes 32 6-13 (1983).
16. S.G. LAYCHOCK, Cell Calcium 3 43-54 (1982).
17. K.L. KELLY and S.G. LAYCHOCK, Prostaglandins 21 759-769 (1981).
18. R.S. SPANGLER and S.B. PEK, Adv. Prostaglandin Thromboxane Leukotriene Res. 12 291-297 (1983).
19. R. KATO, S. YAMAMOTO, T. NAKADATE and T. NAKAKI, Adv. Prostaglandin Thromboxane Leukotriene Res. 12 265-270 (1983).
20. S. METZ, M. VanROLLINS, R. STRIFE, W. FUJIMOTO, and R.P. ROBERTSON, J. Clin. Invest. 71 1191-1205 (1983).
21. S. YAMAMOTO, T. NAKADATE, T. NAKAKI, K. ISHII and R. KATO, Eur. J. Pharmacol. 78 225-227 (1982).
22. J. TURK, N. KOTAGAL and M. GREIDER, Diabetes 32 (Suppl. 1) 139A (1983).
23. V. GRILL, Am. J. Physiol. 240 E24-E31 (1981).
24. J.P. ASHBY and D. SHIRLING, Diabetologia 21 230-234 (1981).
25. S. PEK, Nuclear Medicine: Endocrinology, B. ROTHFELD, ed., pp. 122-140, Lippincott, Philadelphia (1978).
26. F.A. FITZPATRICK and G.L. BUNDY, Proc. Nat. Acad. Sci. USA 75 2689-2693 (1978).
27. G.W. SNEDECOR and W.G. COCHRAN, Statistical Methods, p. 293, Iowa State Press, Ames (1967).
28. M. HAMBERG, Biochem. Biophys. Res. Commun. 49 720-726 (1972).

29. S.B. PEK and M.F. WALSH, Proc. Nat. Acad. Sci. USA (in press) (1984).
30. S.A. METZ, W.Y. FUJIMOTO and R.P. ROBERTSON, Life Sci. 32 903-910 (1983).
31. J.R. VANE, Nature New Biol. 231 232-235 (1971).
32. R.N. BROGDEN, R.C. HEEL, T.M. SPEIGHT and G.S. AVERY, Drugs 18 417-438 (1979).
33. R.O. MORGAN and S.B. PEK, Diabetes 31 (Suppl. 2) 25A (1982).
34. M. R. MacADAMS, S.B. PEK and W.E.M. LANDS, Endocrinology 114 (in press) (1984).
35. R.P. ROBERTSON, Diabetes 32 231-234 (1983).
36. H.R. MORRIS, P.J. PIPER, G.W. TAYLOR and J.R. TIPPINS, Prostaglandins 19 371-383 (1980).
37. R.F. MYERS and M.I. SIEGEL, Biochem. Biophys. Res. Commun. 112 586-594 (1983).