Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 743-749

EPOXYEICOSATRIENOIC ACIDS STIMULATE GLUCAGON AND INSULIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS

J.R. Falck[1], S. Manna[1], J. Moltz[2], N. Chacos[3] and J. Capdevila[3*]

Departments of Molecular Genetics[1], Physiology[2] and Biochemistry[3]
The University of Texas, Health Science Center at Dallas, Dallas, Texas 75235

Received June 17, 1983

Summary: Metapyrone and eicosatetraynoic acid but not indomethacin are effective inhibitors of the secretory response of isolated rat pancreatic islets to arginine and glucose. Epoxyeicosatrienoic acids, products of the cytochrome P-450-NADPH dependent arachidonic acid epoxygenase activity, are potent and selective mediators for the in vitro release of either insulin or glucagon from preparations of isolated rat pancreatic islets.

Introduction: We and others have provided in vitro evidence for the existence of a new pathway of arachidonic acid metabolism, catalyzed by the NADPH-cytochrome P-450 dependent enzyme system (1-4). This enzyme system functions as an active arachidonic acid "epoxygenase" affording four novel cis-epoxyeicosatrienoic acids (EET's), i.e., 5,6-, 8,9-, 11,12- and 14,15-EET (5). The EET's are potent mediators for the in vitro release of certain peptide hormones such as somatostatin from median eminence nerve terminals (2) and luteinizing hormone from isolated anterior pituitary cells (6).

Recent experiments suggest that glucose induced insulin secretion from isolated rat pancreatic islets is coupled to activation of phospholipases and the release of arachidonic acid (7-12). The effect of exogenously added prostaglandins as well as selective inhibitors of arachidonate metabolism support a possible role for lipoxygenase products as in vitro mediators of

---

* - to whom correspondence should be addressed.

Supported in part by grants from USPHS (NIGMS 16488)[3], the Robert A. Welch Foundation (I-782)[1], (NIH-AM 21163)[2] and the American Diabetes Assoc.[2]

Abbreviations: 5,6-EET: cis-5,6-epoxy-8,11,14-cis-eicosatrienoic acid; 8,9-EET, 11,12-EET and 14,15-EET: the corresponding all cis epoxy-eicosatrienoic acids; 5-HETE: 5-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid; 12-HETE: 12-hydroxy-5,8,14- cis-10-trans-eicosatetraenoic acid; ETYA: 5,8,11,14-cis-eicosatetraynoic acid; HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HPLC: high pressure liquid chromatography.

0006-291X/83 $1.50

glucose induced insulin secretion from isolated rat pancreatic islets (13). Prostaglandins, on the other hand, affect pancreatic hormone secretion as "modulators" of the secretory response (14).

It was of interest therefore to examine the effect of the newly described EET's on the release of insulin and glucagon from preparations of isolated rat pancreatic islets. Herein we present evidence implicating the involvement of the EET's in the *in vitro* release of both insulin and glucagon.

METHODS

Pancreatic islets were obtained from male Sprague-Dawley rats (150-180g) according to (15), modified as previously described (16). All *in vitro* testing of the synthetic EET's and inhibitors consisted of a control and various treatment groups of four assay tubes each containing eight isolated islets. These were preincubated in 1 ml of Medium 199 (Gibco Laboratories, Grand Island, NY) containing 0.1% bovine serum albumin, 5.6 mM glucose, 20 mM HEPES, and 1000 K.I.U. Trasylol. After 60 minutes, the medium was replaced with fresh control medium or medium containing the substance(s) to be tested and incubated for an additional 30 minutes. The medium was removed and frozen for subsequent radioimmunoassay for insulin and glucagon content. Insulin was assayed as in (17), as modified by Herbert *et al.* (18). Glucagon was measured with antiglucagon serum 30K according to Faloona *et al.* (19). The metabolism of exogenous arachidonic acid by isolated rat pancreatic islets was studied as follows: 2×10^2 islets were suspended in 2 ml of Medium 199. After a 5 minute preincubation period at 37°C, the cell suspension was transferred to a flask containing 4 nmols of neat 1-[^{14}C]arachidonic acid (5.9 mCi/mmol). The incubation was continued for 30 minutes under constant mixing. The isolation and chromatographic analysis of the products were done as in (1). Control incubations were performed utilizing heat denatured cells.

The synthetic EET's were prepared from 1-[^{14}C]arachidonic acid (10 mCi/mol) by literature methods (20,21). Immediately prior to use, each EET was purified by reverse phase HPLC as described (5). The required amount of EET was placed in the incubation vessel and, after solvent evaporation, the incubations were initiated by adding the cell suspension.

Chemicals: Arachidonic acid was from Nu Chek Prep. (Elysian, Minnesota); 1-[^{14}C]-arachidonic acid from Amersham (Arlington Heights, Illinois); ETYA was a gift from Hoffmann-La Roche (Nutley, NJ). Indomethacin was from Sigma Chem. Co. (St. Louis, MO.) Metapyrone was from CIBA-GEIGY (Ardsley, N.Y.)

RESULTS AND DISCUSSION

Incubation of isolated rat pancreatic islets with arginine (10 mM) results in an approximate two and three fold stimulation in insulin and glucagon secretion, respectively (Fig. 1-a). The arginine-stimulated release of both hormones is strongly inhibited by both ETYA and metapyrone. The cyclooxygenase inhibitor indomethacin (50 μM) fails to alter the arginine-stimulated release of either glucagon or insulin (22). These inhibitors give essentially identical results for glucose-stimulated insulin release (Fig. 1-b). ETYA is a potent competitive inhibitor of both the cyclooxygenase and

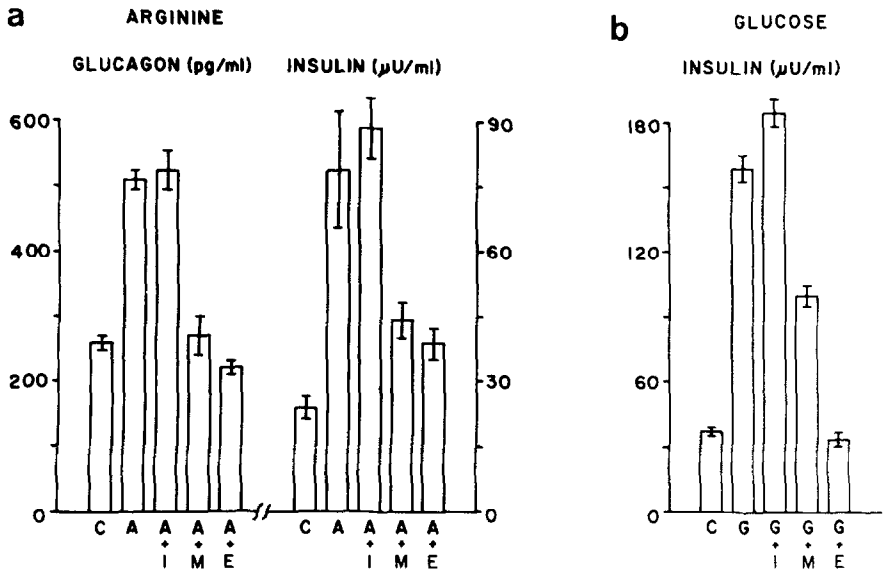

FIGURE 1: Effects of Inhibitors on the Release of Glucagon and Insulin Stimulated by Arginine or Glucose. Isolated rat pancreatic islets were incubated at 37°C for 60 minutes, transferred to incubation vessels containing the different test compounds and then incubated for an extra 30 minutes (see Methods). The values given are the mean ± SEM of eight different incubations. C = controls; A_5 = arginine (10^{-3} M); G = glucose (1.6 x 10^{-2} M); I_5 = indomethacin (5 x 10^{-5} M); M = metapyrone (5 x 10^{-5} M) and E = ETYA (5 x 10^{-5} M). Statistical analysis was done by the Student's t test. Figure 1-a: Arginine stimulated release of glucagon and insulin. p values vs controls are as follows: A < 0.001; A+I < 0.001 for glucagon release; A < 0.025; A+I < 0.01 for insulin release. Figure 1-b glucose stimulated release of insulin. p values vs controls are as follows: G < 0.001; G+I < 0.001; G+M < 0.001. G+M vs G p < 0.001.

lipoxygenase enzyme systems. In experiments to be reported elsewhere (23), we have shown that ETYA, at the concentrations used here, is an effective inhibitor of the cytochrome P-450 catalyzed oxygenation of arachidonic acid. Metapyrone has been shown to be a powerful and selective inhibitor of cytochrome P-450 function in general (24) and of the cytochrome P-450 dependent arachidonic acid oxygenase reactions in particular (1). Although the interpretations of inhibition experiments involving whole cell preparations are limited by the possibility of unknown side effects, these results suggest a role for a metabolite(s) of the cytochrome P-450 dependent pathway in the stimulatory effects of both arginine and glucose in the release of the described hormones from our pancreatic islet preparations. In agreement with (8), incubation of arachidonic acid (5 μM) with isolated islets results in a two-fold increase in the release of insulin and in a smaller but significant

TABLE I

Effect of Arachidonic Acid and 5- and 12-HETE on the Release of Insulin and Glucagon from Isolated Pancreatic Islets.

Experiment	Additions	Insulin(μU/ml)	%	Glucagon(pg/ml)	%
1	Controls	7 ± 0.5	100	126 ± 7	100
	Arachidonic	16 ± 0.4[a]	229	166 ± 7[b]	132
	Arachidonic + ETYA	9 ± 0.6	129	126 ± 6	100
2	Controls	15 ± 2	100	94 ± 6	100
	5-HETE	11 ± 1	73	170 ± 11[c]	181
	12-HETE	14 ± 2	93	210 ± 15[d]	223

The different test compounds were added at the following final concentrations: arachidonic acid 5×10^{-6} M; ETYA 5×10^{-5} M; 5- and 12-HETE 10^{-6} M. The values given are the mean ± SEM of eight different incubations. p values vs controls are as follows: a < 0.001; b < 0.05; c < 0.025; d < 0.005 (Student's t test). None of the remaining values are significantly different from control values.

increase in glucagon release (33% over control values), Table I. Both effects are abolished in the presence of ETYA, suggesting that a metabolite(s) of arachidonic acid was responsible for the increases noted. Under these experimental conditions synthetic 5- and 12-HETE (tested at 10^{-6} M) produces a slight inhibition of the basal level of insulin release and increases glucagon release approximately 80 and 220%, respectively (Table I). Lower concentrations of 5- and 12-HETE failed to significantly alter the basal levels of either hormone. The inhibitory effects of 5- and 12-HETE on insulin release are unexpected; published data, based on the effects of inhibitors of arachidonic acid oxidation in a similar *in vitro* system, have suggested a role for lipoxygenase products in the glucose-induced stimulation of insulin release from isolated rat pancreatic islets (13). This discrepancy is presently unresolved.

The EET's produced by the cytochrome P-450 dependent epoxygenase do not contain a conjugated triene functionality in their molecular structure and are therefore chemically distinct from the leukotriene group (8,25). Table II shows the effect of each of the four synthetic EET's (10^{-6} M) on the release of insulin and glucagon from isolated rat pancreatic islets. Under our

TABLE II

Effect of Epoxygenase Products on the Release of Insulin and Glucagon from Isolated Rat Pancreatic Islets.

	Insulin (μU/ml)	%	Glucagon (pg/ml)	%
Control	24.8 ± 4	100	193 ± 23	100
5,6-EET	49.3 ± 9[a]	199	208 ± 51	108
8,9-EET	26.6 ± 7	107	379 ± 89[b]	196
11,12-EET	26.7 ± 7	108	565 ± 56[c]	293
14,15-EET	24.7 ± 7	100	587 ± 66[d]	304

All incubations were done as described in Methods. The different EET's were added at 10^{-6} M final concentration. The values shown are the mean ± SEM of at least 5 different experiments each of which consisted of eight duplicate incubations. p values vs the corresponding controls are as follows: a < 0.01; b < 0.05; c < 0.005; d < 0.005. Statistical analysis included one way analysis of variance and Student Neuwnan-Keuls Multiple Range Test.

experimental conditions, only 5,6-EET produces a clear increase in the amount of insulin released (199% of control values). The effect of 5,6-EET is selective for insulin; no significant changes in basal glucagon release are observed (Table II). While 8,9-, 11,12- and 14,15-EET do not significantly alter the levels of insulin secretion, they do clearly increase glucagon secretion from isolated rat pancreatic islets (Table II). Their efficacy decreases with the distance of the oxido group from the W terminus: 14,15-EET (304% of control values), 11,12-EET (293% of control values) and 8,9-EET (196% of control values).

The effects of the EET's on in vitro pancreatic hormone secretion are, to our knowledge, unique in their selectivity, levels of stimulation and minimum concentration required to elicit response. 5,6-EET and 14,15-EET stimulate the release of insulin and glucagon, respectively, in a concentration dependent manner (Fig. 2). The first significant increase in insulin release is observed at 10^{-8} M 5,6-EET while that for glucagon occurs at approximately 10^{-8} M 14,15-EET. We were unable to determine a saturation point for the dependency of hormone release for either 5,6- or 14,15-EET due to cell toxicity at doses greater than 10^{-5}M. The effects of 8,9- and 11,12-EET on glucagon release from our preparations are also concentration dependent and, in both cases, are qualitatively similar to that of 14,15-EET.

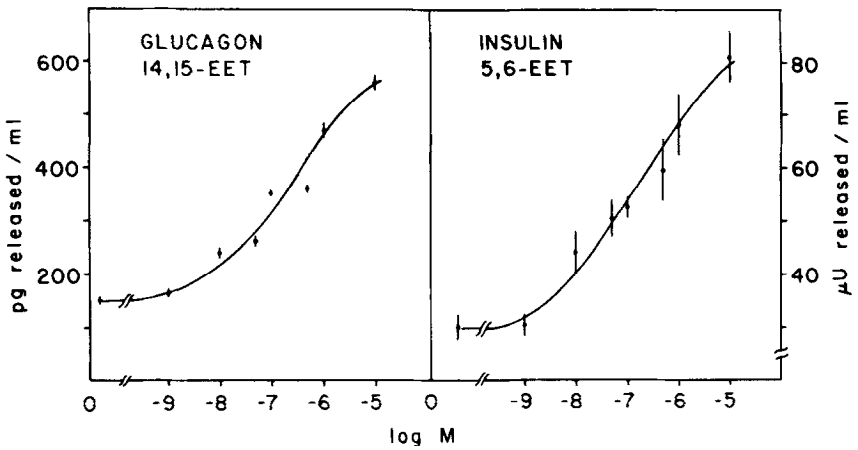

FIGURE 2: Effect of Increasing Concentrations of 14,15-EET and 5,6-EET on the Levels of Glucagon and Insulin Release. Isolated rat pancreatic islets were incubated at 37°C with increasing concentrations of either 5,6-EET or 14,15-EET. After a 30 minute period, the medium was removed and analysed for insulin and glucagon as described in Methods. The values given correspond to the mean ± SEM of at least eight different incubations. The first significant increase in the release of glucagon was obtained at 10^{-8} M 14,15-EET (p vs controls < 0.0005) and that of insulin at 10^{-8} M 5,6-EET (p vs controls < 0.025). (Student's t test).

The mechanism by which the EET's alter the in vitro levels of insulin and glucagon secretion from the isolated islets utilized remains unknown. Once the EET's are formed, they are rapidly hydrated by a cytosolic epoxide hydrolase to form the corresponding vic-dihydroxyacids (26). Hydration of the EET's has been shown to be a route for partial inactivation of the EET's in median eminence fragments as well as in isolated anterior pituitary cells (2,6).

One limitation of our studies is the inability to demonstrate epoxygenation of exogenously added arachidonic acid by our preparation of isolated rat pancreatic islets. Although the reverse phase HPLC chromatograms of the organic soluble products isolated from incubation mixtures containing 2 x 10^{-6} M 1- [^{14}C] arachidonic acid did show small amounts of radioactive material that coeluted with authentic EET standards, the low amounts of radioactive material recovered preclude any meaningful conclusion with regard to their enzymatic formation and chemical identity.

In summary, we have provided evidence suggesting a role for a cytochrome P-450 dependent epoxygenation of arachidonic acid in the secretory response of isolated rat pancreatic islets to glucose and arginine. In addition, we

showed that products of the hemoprotein dependent "epoxygenase" activity are potent and selective mediators for the *in vitro* release of insulin and glucagon from isolated rat pancreatic islets. The *in vivo* significance of this phenomenon during insulin and glucagon release from the endocrine pancreas remains to be established.

REFERENCES

1. Capdevila, J.; N. Chacos; J. Werringloer; R.A. Prough and R.W. Estabrook (1981). Proc. Natl. Acad. Sci. U.S.A. 78, 5362-5366.
2. Capdevila, J.; N. Chacos; J.R. Falck; S. Manna; A. Negro-Vilar and S.R. Ojeda. (1983) Endocrinology in Press.
3. Morrison, A.R. and N. Pascoe (1981). Proc. Natl. Acad. Sci. U.S.A. 78, 7375-7378.
4. Oliw, E.H. and Oates, J.A. (1981). Biochem. Biophys. Acta 666, 327-340.
5. Chacos, N.; J.R. Falck; C. Wixtrom and J. Capdevila (1982). Biochem. Biophys. Res. Comm. 104, 916-922.
6. Snyder, G.D.; J. Capdevila; N. Chacos; S. Manna and J.R. Falck (1983). Proc. Natl. Acad. Sci. U.S.A. in Press.
7. Kelly, K.L. and S.G. Laychock (1981). Prostaglandins 21, 759-769.
8. Metz, S.A.; S. Little; W. Fujimoto and R.P. Robertson (1983) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (B. Samuelsson; R. Paoletti and P. Ramwell, eds.) Vol 12, pp 271-277. Raven Press, New York.
9. Metz, S.A.; Robertson, R.P. and W. Fujimoto (1981). Diabetes 30, 551-557.
10. Yamamoto, S.; T. Nakadate; T. Nakaki; K. Ishii and R. Kato (1982). European J. Pharmacol. 78, 225-227.
11. Kato, R.; S. Yamamoto; T. Nakadate and T. Nakaki (1983) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (B. Samuelsson; R. Paoletti and P. Ramwell, eds.) Vol 12, pp 265-270. Raven Press, New York.
12. Laychock, S.G. (1983). Diabetes 32, 6-13.
13. Metz, S.A.; W. Fujimoto and R.P. Robertson (1982). Endocrinology 111, 2141-2143.
14. Pek, S.B. and M.R. MacAdams (1982) in: Role of Chemical Mediators in the Pathophysiology of Acute Illness and Injury (R. McConn, ed.) pp 291-295. Raven Press, New York.
15. Lacy, P.E. and M. Kostianovsky (1967). Diabetes 16, 35-39.
16. Moltz, J.H.; R.E. Dobbs; S.M. McCann and C.P. Fawcett (1977). Endocrinology 101, 196-202.
17. Yalow, R.S. and S. Berson (1960). J. Clin. Invest. 39, 1157-1175.
18. Herbert, V.; K.S. Lau, C.G. Gottlieb and S.J. Bleicher (1965). J. Clin. Endocrinology Met. 25, 1375-1384.
19. Faloona, G.R. (1973) in: Methods of Investigative and Diagnostic Endocrinology (R.S. Yalow and S. Berson, eds.) Part III, p. 919. North Holland, Amsterdam.
20. Corey, E.J.; A. Marfat; J.R. Falck and J.O. Albright (1980). J. Amer. Chem. Soc. 102, 1433-1435. and cited references.
21. Falck, J.R. and S. Manna (1982). Tetrahedron Letters 23, 1755-1756.
22. Spangler, R.S. and S.B. Peck (1983) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (B. Samuelsson; R. Paoletti and P. Ramwell, eds.) Vol 12, pp 291-297. Raven Press, New York.
23. Capdevila, J.; N. Chacos and R.W. Estabrook (1983) manuscript in preparation.
24. Leibman, K.C. (1969). Mol. Pharmacol. 5, 1-9.
25. Samuelsson, B. (1983). Science 220, 568-575.
26. Chacos, N.; J. Capdevila; J.R. Falck, S. Manna; C. Martin-Wixtrom; S.S. Gill; B.D. Hammock and R.W. Estabrook (1983). Arch. Biochem. Biophys. 223, 639-648.