EXPERIMENTAL BIOLOGY

On the Mechanism of Insulinotropic Action of Hepoxylins: Evidence of a Direct, Glucose-Independent Effect of Hepoxylins B_3 on Insulin Secretion

V. P. Fedotov, N. V. Sadovnikova, I. N. Baranova, L. L. Vasil'eva, T. A. Manukina, and K. K. Pivnitskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 10, pp. 404-406, October, 1995
Original article submitted April 12, 1994

The effects of lipoxygenase metabolites of arachidonic acid, hepoxylin B_3 epimers, on insulin secretion by a culture of isolated islet cells were studied. The effect was assessed at hepoxylin B_3 concentrations of 0.2 to 5.0 μM and different glucose concentrations in the culture medium. Both hepoxylin B_3 epimers were shown to boost the stimulating effect of glucose on insulin secretion. This effect manifests itself at glucose concentrations of 5.5 and 11 mM and disappears at an above normal glucose content in the medium (20 mM). The capacity of hepoxylin B_3 to stimulate the secretion of insulin by a culture of islet cells in a glucose-free medium has also been demonstrated. This direct, not glucose-mediated, insulinotropic effect may serve as proof that the hepoxylins belong to the category of intracellular messengers.

Key Words: *eicosanoids; hepoxylins; pancreatic islet cell culture; insulin secretion*

Hepoxylins (Hx) are a type of eicosanoids, arachidonic acid metabolites that are widely represented in the organism. Hx are formed under the action of 12-lipoxygenases metabolizing arachidonic acid into 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenic acid (12-HPETE), which is then transformed into hydroxyepoxy metabolites dubbed hepoxylins A_3 (HxA_3) and B_3 (HxB_3) [10,11]. Their structural differences consist in the position of the hydroxyl group at the 8th or 10th carbon atom, respectively, and a 9,10-trans-double bond for HxA_3 and an 8,9-cis-double bond for HxB_3 [8]. Each of the Hx types is represented by a pair of epimers differing in the configuration of the hydroxyl group. At present, Hx have been identified in several organs: in the lungs, brain, aortic wall, platelets, and Langerhans' islets [6]. In Langerhans' islets Hx are the principal arachidonic acid metabolites; transformation of exogenous 12-HPETE into HxA_3 and HxB_3 has been shown [7,10,11].

The main biological effect of Hx in Langerhans' islets is the capacity to stimulate glucose-induced secretion of insulin, which has been demonstrated experimentally more than once, including for all 4 synthetic HxA_3 and HxB_3 [11]. The effect was directly related to the glucose present [5]. A parallelism has been demonstrated between circulating glucose, increase of Hx biosynthesis, and the insulin concentration in the blood [4,9,12,14]. Thus, Hx are now regarded as endogenous regulators of insulin secretion.

In this work we investigated the effect of HxB_3 on the secretion of insulin by cultured pancreatic

Institute of Experimental Endocrinology, Endocrinology Research Center, Russian Academy of Medical Sciences, Moscow (Presented by I. G. Akmaev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0010-1036$12.50

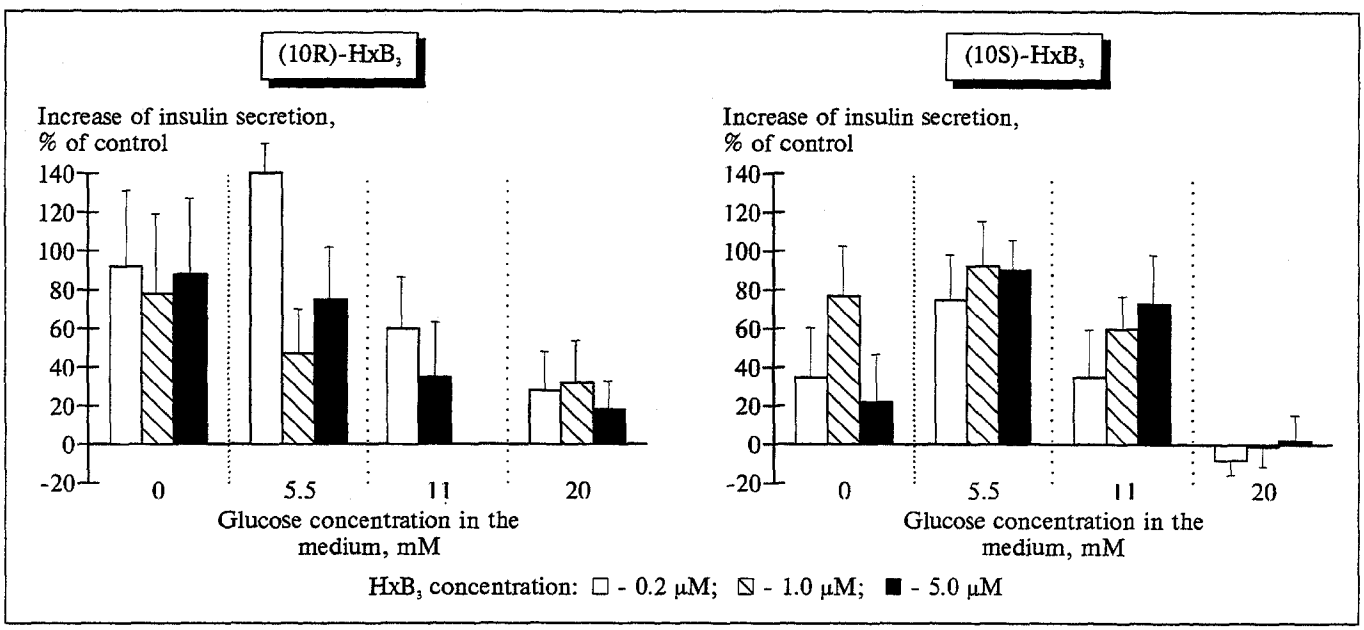

Fig. 1. Effects of HxB_3 on insulin secretion in the presence and absence of glucose in the culture medium (in %).

islet cells at various glucose concentrations in the medium. The possibility of a direct insulinotropic effect of Hx in the absence of glucose was investigated as well.

MATERIALS AND METHODS

Individual (10R)- and (10S)-epimers of HxB_3 in the form of methyl esters obtained by chemical synthesis making use of an original polyacetylene strategy were used in the study [1]. Such synthesis was recently described in detail [15]. HxB_3 epimers were separated and purified by high-performance flash chromatography and preparative thin-layer chromatography. The resultant high-purity samples possessed $[a]D^{25}$ -62.6° ($CHCl_3$) for (10R)-HxB_3 and +61.9° ($CHCl_3$) for (10S)-HxB_3 [15].

The substances were dissolved in absolute ethanol and stored at -30°C. The final concentration of ethanol in the growth medium of isolated islet cells was 0.17%. In control tests such a concentration of ethanol did not change the intensity of insulin secretion by the culture.

The method for obtaining and culturing isolated pancreatic islet cells of newborn rats has been described in detail [2].

A primary culture of islet cells was obtained from rat pups aged 3 to 5 days. The cells were seeded in 96-well Flow Lab. culturing microplates and cultured for 4 days in medium 199 containing 5.5 mM glucose and 10% fetal calf serum (Serva). On the day of the experiment the cultures were divided into groups of 6-8 and the culture medium was replaced with fresh, in which the glucose concentration depended on the purposes of the experiment and varied from 0 to 20 mM.

The cells were preincubated 30 min in this medium, and then Hx was added in concentrations of 0.2 to 5.0 μM and incubation was continued for another 30 min. After the experiment was over, the culture medium was collected and stored at -20°C. The insulin concentration in the samples was radioimmunoassayed using commercial kits (Minsk).

RESULTS

The role of HxB_3 in the manifestation of the insulinotropic effect of glucose was studied at glucose concentrations of 0, 5.5, 11, and 20 mM in the culture medium. Hx were studied in concentrations of 0.2, 1.0, and 5.0 μM. The range of glucose and Hx concentrations, except the zero one, encompassed the range of concentrations studied by other scientists [5,11]. The levels of insulin secretion by the islet cell culture are presented in Table 1. Figure 1 shows the HxB_3-induced changes in the levels of insulin secretion in percent and their relationship to the glucose levels.

Both HxB_3 epimers cause an increase of insulin secretion, which is statistically reliable in the majority of cases, at all glucose concentrations except the maximal (20 mM). The effects of the two HxB_3 epimers are similar, as was demonstrated previously [11]. The absence of a dose dependence of the insulinotropic effect of Hx at all glucose concentrations tested is a new finding, at least in the range of Hx doses tested in our experiments.

The insulinotropic effect of HxB_3 is maximal (49-140% higher than the control) at 5.5 mM glu-

TABLE 1. HxB_3 – Induced Insulin Secretion by a Culture of Islet Cells at Different Glucose Concentrations in the Medium ($M \pm m$)

Group	Hx in medium, μM	Insulin in medium (mU/ml) at glucose concentrations, μM			
		0	5.5	11	20
Control	–	28±7 (6)	75±4 (6)	62±11 (6)	180±15 (8)
(10R) – HxB_3	0.2	54±8* (8)	179±22*** (7)	99±9+ (8)	227±28 (8)
	1.0	50±8* (7)	111±21 (8)	82±13 (7)	237±28 (7)
	5.0	53±6+ (8)	132±23* (7)		214±12 (8)
(10S) – HxB_3	0.2	38±5 (8)	130±22* (8)	83±10 (8)	166±11 (8)
	1.0	50±5+ (8)	144±23+ (8)	98±3** (7)	178±5 (8)
	5.0	35±3 (8)	143±9** (8)	107±16* (6)	185±14 (8)

Note. Number of experiments given in parentheses. *$p<0.05$, **$p<0.01$, ***$p<0.001$, and +$p<0.02$ *vs.* the control.

cose, decreases to 32-72% above the control at 11 mM glucose, and disappears at a still higher glucose concentration (20 mM). These data correspond to the results according to which the stimulating action of Hx is maximal at low basal concentrations of glucose (3 to 5 mM) [6], whereas at a high concentration (23 mM) it may alter and even become inhibitory [5].

The results of an experiment in which islet cells were incubated with HxB_3 in Hanks' solution without glucose appear to be the most interesting. In contrast to relatively weak insulin secretion in the control, a pronounced insulin-stimulating effect of Hx was observed, which was particularly evident for methyl ester (10R)-HxB_3 (79-93% higher than in the control), that is, comparable to the maximal effect at the glucose concentration of 5.5 mM.

Hence, exogenous HxB_3 epimers activate insulin secretion in a culture of islet cells in a medium without glucose and at glucose concentrations approaching the physiological ones. At glucose concentrations higher than normal and under conditions of functional overstrain of β-cells these arachidonic acid metabolites do not express their activity upon exogenous administration.

Glucose is known to promote an increase of the intracellular pool of endogenous Hx [4,12,14], and this evidently speeds up the secretory processes in β-cells. Hx-induced alteration of the level of cytosolic calcium in islet cells may be a factor in this [6]. For this reason, Hx are regarded as second messengers or as factors interacting with them [3,13]. In such a case the direct, not glucose-mediated, insulinotropic effect of HxB_3 may serve to confirm this concept, while the decrease in the insulin-stimulating action of exogenous Hx at ascending glucose concentrations is easy to explain in light of the rise of endogenous Hx concentrations in parallel with the increase of glucose concentrations.

REFERENCES

1. K. K. Pivnitskii, *Zh. Vsesoyuz. Khim. Obshch. im. D. I. Mendeleeva,* **36**, 418-422 (1991).
2. V. P. Fedotov, I. S. Komolov, N. V. Sadovnikova, and V. G. Maslennikova, *Probl. Endokrinol.,* № 4, 57-60 (1978).
3. S. Bevan and J. N. Wood, *Nature,* **238**, 20 (1987).
4. S. Metz, *Proc. Nat. Acad. Sci. USA,* **82**, 198-202 (1985).
5. M. H. Nathan and S. Belbez Pek, *Prostaglandins Leukot. Essent. Fatty Acids,* № 40, 21-25 (1990).
6. C. R. Pace-Asciak, *Gen. Pharmacol.,* **24**, 805-810 (1993).
7. C. R. Pace-Asciak and S. Asotra, *Free Radic. Biol. Med.,* 7, 409-433 (1989).
8. C. R. Pace-Asciak, E. Granstrom, and B. Samuelsson, *J. Biol. Chem.,* **258**, 6835-6840 (1983).
9. C. R. Pace-Asciak, S. P. Lee, and J. M. Martin, *Biochem. Biophys. Res. Commun.,* **147**, 881-884 (1987).
10. C. R. Pace-Asciak and J. M. Martin, *Prostaglandins Leukot. Med.,* **16**, 173-180 (1984).
11. C. R. Pace-Asciak, J. M. Martin, and E. J. Corey, *Prog. Lipid Res.,* **25**, 625-628 (1986).
12. C. R. Pace-Asciak, J. M. Martin, E. J. Corey, and W.-G. Su, *Biochem. Biophys. Res. Commun.,* **128**, 942-946 (1985).
13. D. Piomelli, A. Vorterra, N. Dale, *et al., Nature,* **328**, 38-43 (1987).
14. J. Turk, J. R. Colca, N. Kotagal, and M. L. McDaniel, *Biochim. Biophys. Acta.,* **794**, 125-136 (1984).
15. L. L. Vasiljeva, T. A. Manukina, P. M. Demin, *et al., Tetrahedron,* **49**, 4099-4106 (1993).