Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 78-85

INSULINOTROPIC EFFECT OF THE TUMOR PROMOTER TELEOCIDIN IN ISOLATED PANCREATIC ISLETS

Satoshi Yamamoto, Teruo Nakadate, Hirota Fujiki[1] and Ryuichi Kato

Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, and [1]National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 160, Japan

Received September 26, 1983

SUMMARY: A tumor promoter teleocidin induced insulin secretion from isolated pancreatic islets in a concentration-dependent manner. The teleocidin-induced secretion was inhibited by p-bromophenacyl bromide, nordihydroguaiaretic acid, 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone, but not by indomethacin. Insulinotropic concentrations of teleocidin stimulated 6-keto-prostaglandin $F_{1\alpha}$ release from pancreatic islets. These results suggest that phospholipase A_2 activation and lipoxygenase product(s) are involved in the mechanism of teleocidin-induced insulin secretion.

TPA[2], a phorbol ester tumor promoter, elicits and modulates a variety of biochemical and biological responses in various types of cells (1,2). Recently Fujiki et al., reported that teleocidin (3) and dihydroteleocidin B (4), a catalytically hydrogenated derivative of teleocidin B, which are structurally different from the phorbol esters, have as strong tumor promoting activity as TPA on mouse skin. Moreover, teleocidin and dihydroteleocidin B share many biological or biochemical actions with TPA (5). In addition, teleocidin and dihydroteleocidin B inhibit the specific binding of phorbol ester to membrane receptors at a potency similar to that of TPA (6-8), indicating

[1]To whom correspondence should be addressed.

[2]The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; BPB, p-bromophenacyl bromide; NDGA, nordihydroguaiaretic acid; BW755C, 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline; AA861, 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PG, prostaglandin

0006-291X/83 $1.50

that phorbol ester and teleocidin bind to the same receptor and thus produce similar biological effects. One of the biological effects of TPA is a stimulation of insulin secretion from pancreatic B cells (9-12). Therefore, in this study, we examined the effect of teleocidin on insulin secretion in isolated pancreatic islets in comparison with that of TPA (12).

MATERIALS AND METHODS

Collagenase (Type V), crystallized and lyophilized bovine and human serum albumin, indomethacin, NDGA and TPA were purchased from Sigma Chemical Company, St. Louis, MO, and BPB from Wako Pure Chemical Ind. Ltd., Osaka, Japan. Radioimmunoassay kits for 6-keto-$PGF_{1\alpha}$ and insulin were obtained from New England Nuclear, Boston MA and Dainabot RI Institute Co., Tokyo, Japan, respectively. BW755C and AA861 were gifts from The Wellcome Research Laboratories, Beckenham, Kent, United Kingdom and Takeda Chemical Industries Ltd., Osaka, Japan, respectively.

Male Wistar rats weighing 300 to 350 g were used. Pancreatic islets were isolated as described previously (13). Isolated pancreatic islets were transferred into a Teflon meshed basket in a flask containing 0.5 ml of 95% O_2 and 5% CO_2 saturated Krebs-Ringer bicarbonate solution (pH 7.4) of the following composition (mM): 120 NaCl, 4.8 KCl, 25.5 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.7 $CaCl_2$ and 3.3 glucose supplemented with 0.2% crystallized and lyophilized bovine serum albumin. Islets were preincubated by shaking at 90 strokes/min for 30 min at 37°C. After preincubation, the medium was changed to a new solution containing the indicated concentration of glucose and various agents. Incubation was continued for another 60 min to measure insulin release. To determine the effects of various inhibitors, islets were preincubated for 30 min with indomethacin, for 20 min with NDGA, BW755C or AA861, and for 7 min with BPB. The above drugs except BPB were added to the final incubation medium as well as to the preincubation medium. Released insulin in the medium was assayed by radioimmunoassay using porcine insulin as a standard.

6-Keto-$PGF_{1\alpha}$ release from isolated pancreatic islets was measured by incubating islets (40 to 60 islets/basket) at 37°C for 60 min in Krebs-Ringer bicarbonate solution supplemented with 3.3 mM glucose and 0.1% crystallized and lyophilized human serum albumin (14). 6-Keto-$PGF_{1\alpha}$ released into the medium was determined by radioimmunoassay. Cross reactivity of antiserum was as follows (6-keto-$PGF_{1\alpha}$ = 100%); $PGF_{1\alpha}$ 2.7%, PGE_1 0.9%, $PGF_{2\alpha}$ 1%, PGE_2 0.3%, PGA_1 <0.01%, PGA_2 <0.01%, PGD_2 0.2%, PGB_2 <0.001%, thromboxane B_2 0.3%, 13,14-dihydro-15-keto $PGF_{2\alpha}$ 0.01%.

RESULTS

Since teleocidin, which we used, is a mixture of teleocidin A and teleocidin B, we expressed the concentration of teleocidin in ng/ml instead of μM. Although the molar concentration of

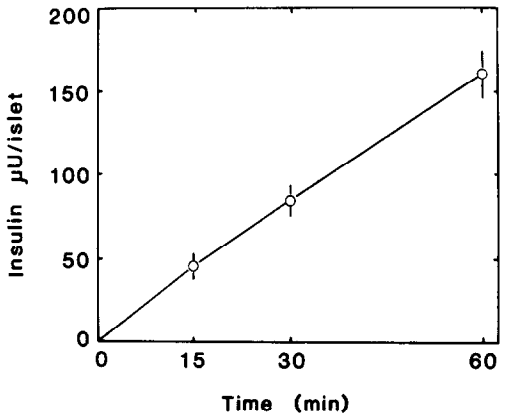

Fig. 1. Time course of insulin secretion from isolated pancreatic islets incubated with teleocidin. Isolated pancreatic islets were incubated with 450 ng/ml of teleocidin in the presence of 3.3 mM glucose at 37°C for the indicated time period. Insulin released into the medium was assayed by radioimmunoassay. Each value represents mean ± S.E. (n=5).

teleocidin depends on the proportion of the content of the above two chemicals, 450 ng/ml corresponds nearly to 1 μM.

Effect of teleocidin on insulin secretion from isolated pancreatic islets was examined in a medium whose glucose concentration was 3.3 mM, i.e. a subthreshold concentration to evoke insulin secretion. Teleocidin (450 ng/ml) induced a noticeable insulin secretion from isolated islets (Fig. 1 and 2). The secretion increased almost linearly during the 60 min

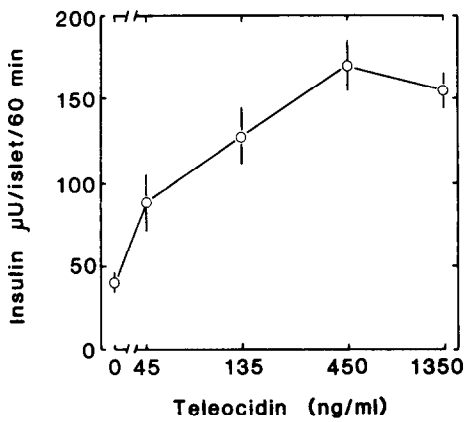

Fig. 2. Effect of teleocidin on insulin secretion from isolated pancreatic islets. Isolated pancreatic islets were incubated with the indicated concentrations of teleocidin in the presence of 3.3 mM glucose at 37°C for 60 min. Insulin released into the medium was assayed by radioimmunoassay. Each value represents mean ± S.E. (n=5).

observation period (Fig. 1). In the same experimental condition, basal insulin release (in the absence of teleocidin) was 47 ± 7 μU/islet/60 min.

Fig. 2 shows the effect of various concentrations of teleocidin on insulin secretion. Teleocidin caused insulin secretion in a concentration-dependent manner. The range of concentration to evoke insulin secretion was similar to that of TPA (12).

Teleocidin-induced insulin secretion was inhibited by a phospholipase A_2 inhibitor, BPB (Table 1). The secretion was also inhibited by NDGA, BW755C and AA861 which are known to inhibit lipoxygenase or both lipoxygenase and cyclooxygenase (15-17). A selective cyclooxygenase inhibitor, indomethacin, however, failed to affect the teleocidin-induced insulin secretion. The concentration of indomethacin (10 μM) we used is sufficient to elicit complete inhibition of prostaglandin formation in pancreatic islets (14).

Table 1
Effects of phospholipase A_2-, cyclooxygenase- and lipoxygenase-inhibitors on teleocidin-induced insulin secretion from isolated pancreatic islets[a)]

	Insulin[b)] μU/islet/60 min
None	56 ± 5 (n=8)*
Teleocidin[c)]	178 ± 13 (n=8)
Teleocidin + BPB 100 μM	97 ± 9 (n=4)*
Teleocidin + Indomethacin 10 μM	165 ± 6 (n=8)
Teleocidin + NDGA 100 μM	59 ± 5 (n=8)*
Teleocidin + BW755C 200 μM	85 ± 13 (n=4)*
Teleocidin + AA861 100 μM	94 ± 7 (n=8)*

a) Glucose concentration of the medium was 3.3 mM.
b) Mean ± S.E.
c) 450 ng/ml
* $p<0.01$ vs Teleocidin

Table 2
Effect of teleocidin on 6-keto-$PGF_{1\alpha}$ release from isolated pancreatic islets[a)]

	6-keto-$PGF_{1\alpha}$[b)] pg/islet/60 min
None	1.79 ± 0.30 (n=7)
Teleocidin 45 ng/ml	3.21 ± 0.27 (n=7)**
Teleocidin 450 ng/ml	3.58 ± 0.52 (n=7)*

a) Glucose concentration of the medium was 3.3 mM.
b) Mean ± S.E.
* $p<0.05$, ** $p<0.01$ vs None

Table 2 shows the effect of teleocidin on 6-keto-$PGF_{1\alpha}$ release from isolated pancreatic islets. Insulinotropic concentrations of teleocidin significantly enhance 6-keto-$PGF_{1\alpha}$ release from isolated pancreatic islets.

DISCUSSION

The present results demonstrate that teleocidin, an indole alkaloid tumor promoter, is able to stimulate insulin secretion from isolated pancreatic islets in a medium whose glucose concentration is at subthreshold level to evoke insulin secretion. A phorbol ester tumor promoter, TPA, which is structurally unrelated to teleocidin, also exerts a potent insulinotropic effect in the absence or in subthreshold concentrations of glucose (10,12). The concentrations of teleocidin required to induce insulin secretion were similar to those of TPA (12). We have recently shown the possible involvement of phospholipase A_2 activation in the mechanism of insulin secretion induced by glucose (14,18,19,20) and some other insulin secretagogues (20) including TPA (12). It has been reported that, like TPA, teleocidin stimulates arachidonic acid release from membrane phospholipids and, as a consequence, enhances prostaglandin release in several types of cells (6,21,22). The release of 6-keto-$PGF_{1\alpha}$ from isolated pancreatic

islets was also stimulated by teleocidin, and the insulinotropic effect of teleocidin was inhibited by a phospholipase A_2 inhibitor, BPB. Therefore, it is highly possible that teleocidin actually stimulates phospholipase A_2 in pancreatic islet and subsequently augments arachidonic acid metabolism either through cyclooxygenase or lipoxygenase pathway. It is known that pancreatic islet is capable of producing lipoxygenase products (23,24) as well as cyclooxygenase products (24,25). It has recently been proposed that lipoxygenase product(s) rather than cyclooxygenase product(s) may play an important role in the mechanism of insulin secretion caused by glucose (18,20,23,26,27) and some other secretagogues (19,20,26,28) including TPA (12). Our recent findings further suggested the significance of 5-lipoxygenase system in the secretory mechanism of insulin (23). The results presented here clearly show that teleocidin-induced insulin secretion was markedly suppressed by lipoxygenase inhibitors. Although these lipoxygenase inhibitors may more or less inhibit cyclooxygenase, the fact that a selective cyclooxygenase inhibitor, indomethacin, failed to inhibit insulin secretion indicates an involvement of lipoxygenase product(s) in teleocidin-induced insulin secretion. Therefore, the mechanism underlying the teleocidin-induced insulin secretion appears to be common to that of TPA. A significant contribution of lipoxygenase product(s) in the mechanism of induction of ornithine decarboxylase and tumor promotion by TPA in mouse epidermis is also postulated recently (29-33). Although it is still preliminary, similar contribution of lipoxygenase product(s) in teleocidin-caused induction of ornithine decarboxylase is also suggested (unpublished data). Thus, it is of interest to elucidate the role of lipoxygenase product(s) in these cellular responses, such as insulin secretion, ornithine

decarboxylase and tumor promotion. Further investigations are now underway.

REFERENCES

1. Boutwell, R. K. (1978) in Carcinogenesis, a Comprehensive Survey, Vol. 2, pp. 49-58 (Slaga, T. J., Sirak, A. and Boutwell, R. K., eds.) Raven Press, New York.
2. Blumberg, P. M. (1981) CRC Crit. Rev. Toxicol. 9, 199-234.
3. Fujiki, H., Suganuma, M., Matsukura, N., Sugimura, T. and Takayama, S. (1982) Carcinogenesis 3, 895-898.
4. Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T. and Moore, R. E. (1981) Proc. Natl. Acad. Sci. USA 78, 3872-3876.
5. Sugimura, T. (1982) Gann 73, 499-507.
6. Umezawa, K., Weinstein, I. B., Horowitz, A., Fujiki, H., Matsushima, T. and Sugimura, T. (1981) Nature 290, 411-413.
7. Solanki, V. and Slaga, T. J. (1981) Proc. Natl. Acad. Sci. USA 78, 2549-2553.
8. Schmidt, R., Adolf, W., Marston, A., Roeser, H., Sorg, B., Fujiki, H., Sugimura, T., Moore, R. E. and Hecker, E. (1983) Carcinogenesis 4, 77-81.
9. Virgi, M. A. G., Steffes, M. W. and Estensen, R. D. (1978) Endocrinology 102, 706-711.
10. Malaisse, W. J., Sener, A., Herchuelz, A., Carpinelli, A. R., Poloczek, P., Winand, J. and Castagna, M. (1980) Cancer Res. 40, 3827-3831.
11. Deleers, M., Castagna, M. and Malaisse, W. J. (1981) Cancer Lett. 14, 109-114.
12. Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. and Kato, R. (1982) Biochem. Biophys. Res. Commun. 105, 759-765.
13. Nakaki, T., Nakadate, T., Ishii, K. and Kato, R. (1981) J. Pharmacol. Exp. Ther. 216, 607-612.
14. Yamamoto, S., Ishii, M., Nakadate, T. and Kato, R. (1983) Biochem. Biophys. Res. Commun. 114, 1023-1027.
15. Hamberg, M. (1976) Biochim. Biophys. Acta 431, 651-654.
16. Higgs, G. A., Flower, R. J. and Vane, J. R. (1979) Biochem. Pharmacol. 28, 1959-1961.
17. Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S. and Shiraishi, M. (1982) Biochim Biophys. Acta 713, 470-473.
18. Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. and Kato, R. (1982) Eur. J. Pharmacol. 78, 225-227.
19. Yamamoto, S., Nakaki, T., Nakadate, T. and Kato, R. (1982) Eur. J. Pharmacol. 86, 121-124.
20. Kato, R., Yamamoto, S., Nakadate, T. and Nakaki, T. (1983) Adv. Prostaglandin, thromboxane and Leukotriene Res. 12, 265-270.
21. Horowitz, A. D., Fujiki, H., Weinstein, I. B., Jeffrey, A., Okin, E., Moore, R. E. and Sugimura T. (1983) Cancer Res. 43, 1529-1535.
22. Sakamoto, H., Terada, M., Fujiki, H., Mori, M., Nakayasu, M., Sugimura, T. and Weinstein, I. B. (1981) Biochem. Biophys. Res. Commun. 102, 100-107.
23. Yamamoto, S., Ishii, M., Nakadate, T., Nakaki, T. and Kato, R. (1983) J. Biol. Chem. in press.
24. Kelly, K. L. and Laychock, S. G. (1981) Prostaglandins 21, 759-769.
25. Evans, M. H., Pace, C. S. and Clements, Jr. R. S. (1983) Diabetes 32, 509-515.

26. Metz, S. A., Fujimoto, W. Y. and Robertson, R. P. (1982) Endocrinology 111, 2141-2143.
27. Metz, S., Van Rollins, M., Strife, R., Fujimoto, W. and Robertson, R. P. (1983) J. Clin. Invest. 71, 1191-1205.
28. Metz, S. A., Fujimoto, W. Y. and Robertson, R. P. (1983) Life Sci. 32, 903-910.
29. Nakadate, T., Yamamoto, S., Ishii, M. and Kato, R. (1982) Cancer Res. 42, 2841-2845.
30. Nakadate, T., Yamamoto, S., Ishii, M. and Kato, R. (1982) Carcinogenesis 3, 1411-1414.
31. Nakadate, T., Yamamoto, S., Iseki, H., Sonoda, S., Takemura, S., Ura, A., Hosoda, Y. and Kato, R. (1982) Gann 73, 841-843.
32. Fischer, S. M., Mills, G. D. and Slaga, T. J. (1982) Carcinogenesis 3, 1243-1245.
33. Kato, R., Nakadate, T., Yamamoto, S. and Sugimura, T. (1983) Carcinogenesis in press.