POTENTIATION OF INSULIN SECRETORY RESPONSE OF RATS BY CELL WALL SKELETON EXTRACTED FROM BCG CELL WALL

Tsutomu KAWATA and Koichi ITAYA
Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

In the previous paper (1), we reported that BCG cells injected into rats as Freund's complete adjuvant (FCA) enhanced insulin release stimulated by secretagogues, and insulin thus released attenuated some metabolic effects of the agonist. BCG is known as an immunopotentiator. It also has been known that BCG cell walls are as active as viable BCG in the immunotherapy of a certain guinea pig hepatoma (2, 3). Azuma et al. isolated the components, cell wall skeleton (CWS), effective in tumor therapy from BCG cell wall (4). Therefore, it seems possible that an immunosuppressant may inhibit the insulin-releasing action of FCA, and CWS may be one of the components that are responsible for stimulation of insulin secretion. In this study, we used azathioprine injection or an irradiation procedure to suppress the immunological activity of the rats, and CWS was used to clarify the latter possibility.

In the first experiment, rats were injected i.p. with azathioprine dissolved in saline containing 0.1% methyl cellulose at a dose of 40 mg/kg/day for 4 days before the experiment. FCA was injected 24 hr after the last injection of the inhibitor. Twenty-four hr after the injection of FCA, the stimulatory action of isoproterenol on the plasma level of insulin was tested. However, the immunosuppressant did not inhibit the supersensitizing action of FCA on insulin secretion stimulated by isoproterenol (data not shown). FCA also enhanced insulin release stimulated by isoproterenol even in the irradiated rats as effectively as in the control rats (Fig. 1). These two experiments suggest that the stimulatory action of FCA on insulin secretion stimulated by isoproterenol was not mediated by its immunopotentiating action. However, we cannot rule out any effects due to the activation of the immunological system by FCA on insulin secretion since irradiation itself enhanced insulin release stimulated by isoproterenol as shown in Fig. 1.

By repeated treatment of the BCG cell wall with proteolytic enzymes such as trypsin and chymotrypsin, followed by extraction with organic solvents, an insoluble residue, CWS, was isolated (4). CWS is a polymeric mycolic acid-arabinogalactan-mucoprotein complex. In the next experiment, CWS was injected into rats instead of FCA 24 hr before the injection of isoproterenol. CWS was suspended in Freund's incomplete adjuvant
Fig. 1. Effect of FCA on isoproterenol-stimulated release of insulin in the irradiated rats (800 Rad). FCA was injected i.p. (1 ml/100 g) 24 hr before injection of isoproterenol, which was injected (200 µg/kg) at 0-time. Mean±S.E.M. from 3 observations. ○—○: normal rats, △—△: irradiated rats, ●—●: FCA-injected rats, ■—■: irradiated and FCA-injected rats.

(FIA). Figure 2 shows that treatment of rats with CWS induced the supersensitivity in insulin secretion as effectively as that with FCA. We also found that poly A:U which has adjuvant activity decreased the plasma level of glucose elevated by epinephrine (data not shown), suggesting that it may induce the supersensitivity in insulin secretion; and the insulin thus released may attenuate the action of epinephrine to increase the plasma level of glucose. It seems possible that any reagent which has adjuvant activity may be able to induce the supersensitivity in insulin secretion. However, we reported that reserpine which has no adjuvant activity induced the supersensitivity to the insulin-releasing action of isoproterenol (6). This with the result shown in Fig. 1 and that with azathioprine shows that the two activities of FCA or CWS, that is, the adjuvant and the supersensitizing one, are independent of each other.

Rook and Stewart-Tull reported that peptidoglycolipids such as CWS were lymphocyte mitogens (7). They also studied the binding of adjuvant-active mycobacterial peptidoglycolipids to mammalian plasma membranes (8). Ca ions have been suggested as the critical signal for initiating the mitotic response (9). On the other hand, it was discussed that the calcium flux through the pancreatic B-cell membrane is closely related to the cyclic AMP-dependent insulin secretion (10). Therefore, FCA or CWS may initially act with islet B-cell membranes as an activator of Ca ion influx. More work should be done for the mechanism of CWS actions to be fully understood.

Acknowledgments: We are greatly indebted to Prof. M. Ui for his encouragement during this study. We also thank Dr. M.
Kakinuma, Institute of Immunological Science, Hokkaido University, for his advice in the irradiation examinations and for allowing us to use the apparatus in his laboratory and Dr. I. Azuma in the same Institute for giving us cell wall skeletons. Part of this work was supported by a grant from the Takeda Science Foundation.

REFERENCES


7) Rook, G.A.W. and Stewart-Tull, D.E.S.: The dissociation of adjuvant properties of mycobacterial components from mitogenicity, and from the ability to induce the release of mediators from macrophages. Immunology 31, 389–396 (1976)

