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ADP-RIBOSYLATION OF ISOLATED RAT ISLETS OF LANGERHANS

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Incubation of isolated rat islets of Langerhans with [adenine-2,8-^{3}H]NAD^{+} results in rapid incorporation of ^{3}H into acid-insoluble products. The major site of incorporation appears to be the cell membrane. The reaction is inhibited by nicotinamide, an ADP-ribosylation inhibitor, and stimulated by arginine, an ADP-ribose acceptor. The results demonstrate that islet membrane proteins can be ADP-ribosylated in the absence of exogenous ADP-ribosylating agents and suggest that ADP-ribosylation plays a role in pancreatic islet cell function.

A variety of microbial toxins are known to exert their toxic effect on eukaryotic cells through the ADP-ribosylation of specific acceptor sites (1-5). Cholera toxin, for example, activates membrane bound adenylate cyclase by ADP-ribosylating a 42,000 MW protein of the guanine nucleotide regulatory subunit, thereby altering the inhibitory influence of the regulatory subunit on adenylate cyclase (6-8).

Although the action of these toxins demonstrates the existence of regulatory sites that can be modified by external ADP-ribosylating agents, it is not clear that such sites actually undergo modification by endogenous ADP-ribosyltransferases under physiological conditions (9). In the present work, we show that islet cells have the intrinsic capacity to ADP-ribosylate their own membrane proteins, suggesting that ADP-ribosylation may be involved in the regulation of islet cell function.

MATERIALS AND METHODS

Preparation of Islets - Islets from adult Lewis rats were prepared by the collagenase method of Lacy and Kostianovsky (10), as modified by Amamoo et al.

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(11), and were suspended in Hanks' salts (12) at a concentration of about 1 islet/µl. Intactness of the islets was verified by microscopic examination and by the ability of similar preparations to reverse the diabetic state of streptozotocin-diabetic rats upon intrahepatic implantation (11).

Reaction Conditions - Incubations were conducted in a plastic microtiter plate (Falcon) mounted on a brass box that was clamped to a shaker platform and kept at 37° by the circulation of water. Reaction mixtures had a final volume of 150 µl and contained 40 µl of Hanks' salts, 10 µl of 0.15 M Na-HEPES[1], pH 7.4, and 100 µl of suspended islets, added to start the reaction. Additions to the reaction mixture were made at the expense of Hanks' salts. Reaction mixtures were covered with tape and shaken at 200 rpm. Chemicals were of reagent grade quality, and solutions were prepared with Pyrex-distilled water that was previously deionized.

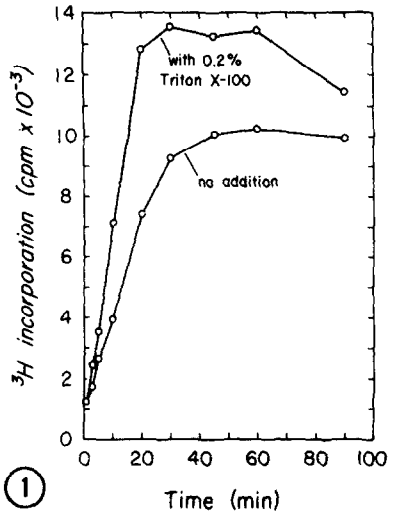
ADP-ribosylation - Reaction mixtures contained 20 µl (2 µCi, 0.6 nmol) of [adenine-2,8-^{3}H]NAD$^+$ in 50% ethanol (New England Nuclear), evaporated in the reaction well before use. Other conditions are described above and in the legends to figures. For sampling, duplicate 10-µl aliquots were transferred to 24-mm Millipore, Type HA filter disks kept on a plastic screen that was heated by a warming tray maintained at 60°. Dried discs were washed 3 times in 500-ml changes of cold, 10% (w/v) trichloroacetic acid, blotted, placed face-up on the bottom of a scintillation vial, overlayed with 10 ml of Aquasol (New England Nuclear), and assayed for radioactivity with a Packard, Model 3310 scintillation counter. Activity is expressed as cpm per 10-µl aliquot and represents the conversion of [adenine-2,8-^{3}H]NAD$^+$ to an acid-insoluble form. A correction was made for the small amount of radioactivity bound to the Millipore filter in the absence of islets.

RESULTS

Figure 1 (lower curve) shows that there was a rapid uptake of ^{3}H into acid-insoluble products when intact islets were incubated with [adenine-2,8-^{3}H]NAD$^+$. Disruption of the islets would be expected to greatly increase the extent of ^{3}H incorporation through the ADP-ribosylation of intracellular proteins and formation of poly(ADP-ribose) (4,5,13-16). However, disruption of the islets with 0.2% Triton X-100 (Fig. 1, upper curve) resulted in an overall increase in ^{3}H incorporation of only 40%, suggesting that most of the ADP-ribosylation activity of islet cells is associated with the cell membrane. Triton X-100 at a concentration of 1% did not further increase the uptake of ^{3}H.

Nicotinamide is a well-known inhibitor of ADP-ribosylation reactions, and the addition of 20 mM nicotinamide to intact islets caused an overall inhibition of 36% under the conditions of Fig. 1 (not shown). In addition, nicotin-

[1]The abbreviation used is HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.



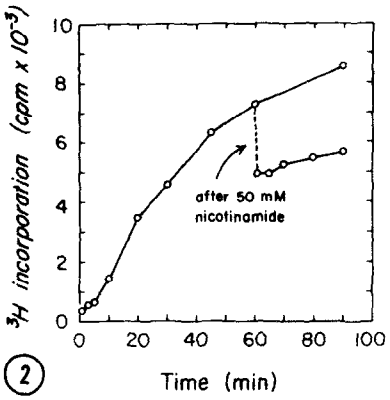


Figure 1 - Incorporation of [adenine-2,8-^{3}H]NAD$^+$ into acid-insoluble products. Islets were incubated at 37° and the acid-insoluble radioactivity determined in 10-µl aliquots as described in text. For the upper curve, 10 µl of 3% Triton X-100 replaced 10 µl of Hanks' salts. Results are means of duplicate experiments.

Figure 2 - Release of radioactivity from ADP-ribosylated islets. Islets were incubated at 37° in two parallel wells and the acid-insoluble radioactivity of 10-µl aliquots determined in one of them (upper curve) as described in text. At 60 min, 20 µl of 375 mM nicotinamide was added to the second well (final volume, 150 µl), and 10-µl aliquots were assayed for acid-insoluble radioactivity at the times indicated (lower curve).

amide had the noteworthy ability to discharge a portion of the incorporated ^{3}H. Fig. 2 shows that, when 50 mM nicotinamide was added to an incubation mixture that was first allowed to incorporate ^{3}H from [adenine-2,8-^{3}H]NAD$^+$ for 60 min, there was an immediate release of 33% of the radioactivity. Table 1 shows that this effect was dependent upon the nicotinamide concentration and that theophylline was more effective than nicotinamide in releasing ADP-ribose from islets. The basis for these effects is still unclear, although they suggest the presence of labile ADP-ribose linkages.

Interestingly, arginine was found to stimulate ^{3}H incorporation from [adenine-2-8-^{3}H]NAD$^+$ (Table 1), suggesting that arginine is a substrate for ADP-ribosylation, as has been found for other ADP-ribosyltransferases (6,17). Arginine is also known to stimulate glucagon and insulin release from islets in a manner that is glucose dependent (18,19).

Table 1 - Release of Radioactivity from ADP-Ribosylated Islets[a]

Compound tested (final concn.)	Radioactivity released (%)
Nicotinamide, 10 mM	4.5
Nicotinamide, 100 mM	40.3
Theophylline, 10 mM	30.3
Arginine, 10 mM	-9.4[b]

[a] Islets were incubated at 37°C with [adenine-2,8,-^{3}H]NAD$^+$ for 60 min as described in text and aliquots (50 µl) transferred to either 10 µl of Hanks' salts (control) or 10 µl of the compound tested. Duplicate 10-µl samples were taken 10 and 20 min later and assayed for acid-insoluble radioactivity. Results are expressed as the percent difference between experimental and control values and are means of duplicate experiments.

[b] Stimulation of ^{3}H incorporation.

DISCUSSION

The present paper demonstrates that intact islets have the ability to ADP-ribosylate their own membrane proteins by means of endogenous ADP-ribosyltransferase(s). This result sugggests that the sites of ADP-ribosylation previously recognized through the action of exogenous microbial ADP-ribosyltransferases may also be involved in physiological regulatory mechanisms, although the identity of the sites that are ADP-ribosylated by microbial and endogenous ADP-ribosyltransferases remains to be established.

Although a number of important eukaryotic cell functions such as protein synthesis and various cyclic AMP-mediated events are known to be affected by ADP-ribosylation (1-5), of particular relevance to the present work is the activity of pertussis toxin, a recently described ADP-ribosyltransferase that has been purified to homogeneity from the culture medium of Bordetella pertussis (20).

When pertussis toxin is injected into rats, it causes a prolonged insulin response to glucose and other insulin secretagogues (21,22) which is also observable with the isolated pancreas and islets of toxin-treated animals (22,23). Because of this effect on insulin release, pertussis toxin was known

only as "islet-activating protein," or "IAP" before its ADP-ribosylating activity was discovered. However, pertussis toxin is now known to catalyze the ADP-ribosylation of a 41,000 MW membrane protein in a variety of intact cells and isolated membranes (24). The 41,000 MW protein appears to be a component of the guanine nucleotide regulatory subunit involved in the regulation of adenylate cyclase, and in the presence of GTP, adenylate cyclase is activated when this protein is ADP-ribosylated (24-29). Although cholera toxin has a similar mode of action, Katada and Ui (24) have shown that the peptides ADP-ribosylated by pertussis toxin and cholera toxin are not the same.

The above differences in toxin specificity point to the existence of multiple regulatory sites for modulating adenylate cyclase activity. Because pertussis toxin can ADP-ribosylate one of these sites as well as promote islet insulin release, the latter two events may be related. The present demonstration in islets of endogenous enzymes that can ADP-ribosylate membrane proteins indicates that such a relationship could exist under physiological conditions.

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