EFFECT OF ALLOXAN ON ISLET TISSUE PERMEABILITY: PROTECTION AND REVERSAL BY NADPH

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SUMMARY

Previous work has shown that incubation of slices of the toadfish islet of Langerhans in a diabetogenic concentration of alloxan increases the permeability of their cell membranes to D-[1-^{14}C]-mannitol, which normally remains in the extracellular space. We have now found that prior incubation of the slices in as little as 10^{-5}M NADPH, which markedly stimulates insulin release, protects the slices against this action of alloxan. Similarly, incubation of islet slices in NADPH after incubation in alloxan reverses the action of the latter. NADP, which produces little or no stimulation of insulin release, does not protect against or reverse the action of alloxan. These results suggest that alloxan may damage the β-cell membrane by acting at or near a site involved in insulin release.

On the basis of a number of studies on the mechanism of the diabetogenic action of alloxan (1-8), we have postulated that alloxan kills the β-cells of the islets of Langerhans by damaging the cell membrane through a reaction with two closely spaced SH groups at or near a sugar binding site. In a parallel series of studies on the mechanism of insulin secretion we showed that the reduced nicotinamide adenine dinucleotides, NADH and NADPH, but not the oxidized forms, markedly stimulate insulin release from intact toadfish islets in vitro and from isolated insulin secretion granules (9-11); these and subsequent findings (12, 13) suggested that the reduced nucleotides may participate in insulin release by reacting with SH groups on the beta cell and beta granule membranes to trigger or facilitate the interaction of the two membranes which occurs during emiocytosis.

In view of this, it seemed possible that the cell membrane SH groups with

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which alloxan reacts may be the same ones with which the reduced nicotinamide adenine dinucleotides react to stimulate insulin release. If so, one would expect that the reduced nucleotides should interfere with the action of alloxan. In keeping with this prediction we have now shown that NADPH protects against and reverses the increase in permeability of toadfish islet cell membranes produced by a diabetogenic concentration of alloxan added in vitro.

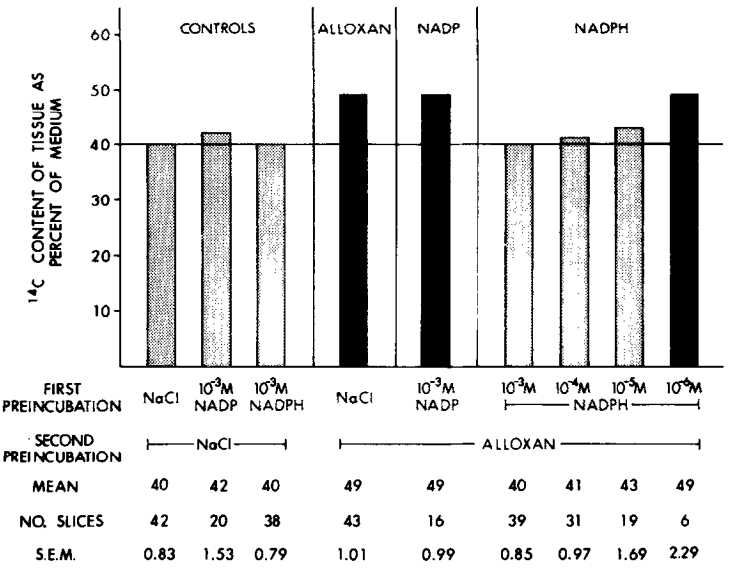
MATERIALS AND METHODS

D-[1-^{14}C]-mannitol with a specific activity of 2.5 mCi/mmole was purchased from the Mallinckrodt Chemical Co. as an alcoholic solution; it was evaporated to dryness and redissolved at a concentration of 7.5 μCi/ml in 0.14 M NaCl containing 10^{-3}M Na_2HPO_4-KH_2PO_4 buffer pH 7.1; this concentration of NaCl has a tonicity equivalent to that of toadfish blood (14). Alloxan was purchased from the Eastman Organic Chemical Co.; just prior to each experiment an isotonic solution was prepared at a concentration of 2.5 x 10^{-4}M exactly as described previously (7). NADPH and NADP were purchased from the Sigma Chemical Co. and dissolved in the buffered saline at the concentrations indicated; NADP was neutralized to pH 7.1 with 1 N NaOH just before use.

As in all of our previous studies, toadfish islets were used because, in contrast to mammals, the islet tissue in this species is segregated into a discrete mass which can be obtained free of pancreatic acinar tissue (15). The islets were decapsulated, sliced into halves or thirds, and transferred to microtubes containing 100 μl of medium as described previously (2). After 15 minutes incubation at 0°C, the slices were rinsed as described previously (4), transferred to a second microtube containing 100 μl of medium, incubated an additional 15 minutes at 0°C, rinsed again, and finally transferred to a third microtube containing 100 μl of D-[1-^{14}C]-mannitol; mannitol was used as an indicator of the integrity of the cell membrane since it is normally restricted to the extracellular compartment. After 60 minutes incubation at 0°C, the slices were removed, rinsed, weighed and homogenized as described previously (3), the ^{14}C contents of the homogenates and incubation media were determined by liquid scintillation spectrometry, and the tissue ^{14}C content (cpm/mg) was expressed as a percentage of the ^{14}C content of the medium (3).

RESULTS

Figure 1 shows that when islet slices were preincubated in two changes of saline and then incubated in D-[1-^{14}C]-mannitol, the ^{14}C content of the tissue was 40% of that in the medium; this is similar to values previously obtained (4, 5), and presumably represents the distribution of mannitol in the extracellular compartment. When islet slices were preincubated in saline and then incubated in 2.5 x 10^{-4}M alloxan before incubation in D-[1-^{14}C]-mannitol, the mannitol space increased to

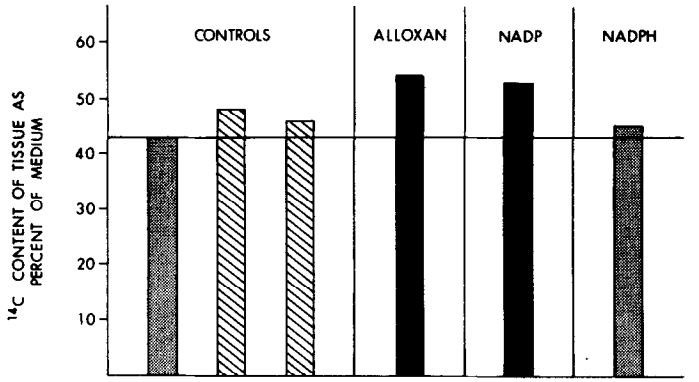


FIRST PREINCUBATION	NaCl	10^{-3}M NADP	10^{-3}M NADPH	NaCl	10^{-3}M NADP	10^{-3}M NADPH	10^{-4}M NADPH	10^{-5}M NADPH	10^{-6}M NADPH
SECOND PREINCUBATION	NaCl			ALLOXAN					
MEAN	40	42	40	49	49	40	41	43	49
NO. SLICES	42	20	38	43	16	39	31	19	6
S.E.M.	0.83	1.53	0.79	1.01	0.99	0.85	0.97	1.69	2.29

Fig. 1 NADPH protection against the increase in islet permeability produced by alloxan. The concentration of alloxan was 2.5×10^{-4}M and the NaCl solution consisted of 0.14 M NaCl in 10^{-3}M Na_2HPO_4-KH_2PO_4, pH 7.1.

49% ($p < 0.001$), indicating that alloxan had damaged the islet cell membranes, thereby permitting mannitol to enter the cells (3). Figure 1 also shows that whereas incubation in 10^{-3}M NADP or NADPH followed by incubation in saline did not significantly modify the permeability of islet tissue to D-[1-^{14}C]-mannitol, incubation of islet slices in 10^{-3}-10^{-5}M NADPH before incubating them in 2.5×10^{-4}M alloxan protected the slices against the action of alloxan. By contrast, 10^{-3}M NADP was ineffective.

Figure 2 shows that 5×10^{-4}M NADPH, but not NADP, likewise reversed the action of alloxan on islet tissue; the mannitol space of islet slices incubated in alloxan followed by incubation in NADPH was significantly lower than that of slices incubated in alloxan followed by saline ($p = 0.004$), and did not differ significantly from that of the controls. This effect of NADPH was observed despite the fact that, in contrast to the results obtained when it was present during the first preincubation



FIRST PREINCUBATION	NaCl			ALLOXAN		
SECOND PREINCUBATION	NaCl	NADP	NADPH	NaCl	NADP	NADPH
MEAN	43	48	46	54	53	45
NO. SLICES	10	11	10	12	12	11
S.E.M.	0.66	1.46	1.41	2.30	1.55	2.05

Fig. 2 NADPH reversal of the increase in islet permeability produced by alloxan. The concentrations of NADP and NADPH were 5 x 10^{-4}M; those of the other reagents were the same as in Figure 1.

period (Fig. 1), when NADPH was present during the second preincubation period (Fig. 2) it, like NADP, produced a small (7%) but barely significant ($p = 0.05$) increase in the mannitol space. While this may seem paradoxical, this phenomenon has also been observed with other protective agents (cf. 8); as pointed out previously (8), it can be explained if NADPH binds at the same membrane site as does alloxan to produce a qualitatively similar but quantitatively lesser effect, and at the same time interferes with the binding of the latter.

DISCUSSION

We have previously shown that tracer doses of [2-^{14}C]-alloxan do not enter toadfish islet cells (1, 2), and that incubation of islet slices in a diabetogenic concentration of alloxan damages the islet cell membranes, thereby increasing their permeability; the uptake of extracellular markers such as mannitol and inulin, and the leakage of insulin and other proteins from the slices, are markedly increased (3).

In view of the ability of various SH-binding reagents to duplicate the action of alloxan (4), and that of various thiols, dithiols and sugars to protect against the action of alloxan and the other SH-binding reagents (3, 5-8), we have proposed that this membrane damage results from a reaction of alloxan with two closely spaced SH groups at or near a sugar binding site (4, 6-8). This hypothesis is supported by a number of studies by other investigators on mammalian islets (cf. 7, 8), but neither our results nor those of others have clearly defined the exact physiological role of the membrane SH groups with which alloxan reacts (cf. 8).

Our hypothesis that the reduced nicotinamide adenine dinucleotides may participate in glucose-stimulated insulin release by reacting with SH groups on the beta cell and beta granule membranes is likewise supported by studies of others using mammalian islets, as well as by our own studies on toadfish islets. The concentrations of the reduced nicotinamide adenine dinucleotides increase during glucose metabolism (16, 17) and when insulin release is stimulated by glucose (16-18) and certain other secretagogues (19). NADH and NADPH stimulate insulin release from intact islets (9, 10, 20) and from isolated insulin secretion granules (11, 21). Several agents which decrease the reduced nucleotide levels of islet tissue interfere with insulin secretion and the latter is restored by increasing the levels of these nucleotides in vitro (20, 22, 23). The presence of SH groups on the β-cell membrane has been demonstrated (4), and their involvement in insulin release has been well documented (e.g., 24); we have likewise shown that SH groups are present in the membrane of the insulin secretion granule and have provided evidence that these too may be involved in insulin release (12, 13). Finally, the reduced nucleotides are known to react with SH groups (25) and have been shown to interact with membranes in other systems (26) as well as with those of the β-cell (10) and the isolated insulin secretion granule (13).

The results presented in this paper clearly show that NADPH protects against and reverses the increase in islet permeability produced by alloxan. Since NADPH does not enter islet cells (10), this finding supports the thesis that alloxan acts on the cell membrane. Furthermore, since NADP does not protect against or reverse the action of alloxan, and since NADPH is likewise more effective than NADP in stimulating insulin release from toadfish islets (9), these results suggest that the insulin releasing action of the reduced nucleotides takes place via the same membrane SH groups with which alloxan reacts, and therefore that the membrane SH groups with which alloxan reacts are somehow involved in insulin release. At the same time, these data do not rule out the possibility that these SH groups are also involved in other cellular functions. For example, in view of the ability of sugars to protect against the action of alloxan (cf. 6, 8), and in view of the involvement of SH groups in islet sugar transport (24), the alloxan-sensitive membrane SH groups may be involved in the latter function as well; for this reason it will be of interest to examine the effect of the nicotinamide adenine dinucleotides on sugar uptake by islet tissue.

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REFERENCES

1. Cooperstein, S. J., and Lazarow, A. (1964) Am. J. Physiol., 207, 423-430.
2. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1964) Am. J. Physiol., 207, 431-435.
3. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1964) Am. J. Physiol., 207, 436-440.
4. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1970) Am. J. Physiol., 219, 503-509.
5. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1971) J. Pharmacol. Exptl. Therap., 176, 42-51.
6. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1973) Am. J. Physiol., 224, 718-722.

7. Watkins, D., and Cooperstein, S. J. (1976) J. Pharmacol. Exptl. Therap., 199, 575-582.
8. Cooperstein, S. J., and Watkins, D. (1977) J. Pharmacol. Exptl. Therap., in press.
9. Watkins, D., Cooperstein, S. J., Dixit, P. K., and Lazarow, A. (1968) Science, 162, 283-284.
10. Watkins, D., Cooperstein, S. J., and Lazarow, A. (1971) Endocrinology, 88, 1380-1384.
11. Watkins, D. (1972) Endocrinology, 90, 272-276.
12. Watkins, D., and Moore, M. (1974) Endocrinology, 96, 485-491.
13. Watkins, D., and Moore, M. (1977) Endocrinology, 100, 1461-1467.
14. Green, J. W., and Hoffman, J. F. (1953) Biol. Bull., 105, 289-295.
15. Diamare, V. (1899) Intern. Monatsschr. Anat., 16, 155-209.
16. Panten, U., Poser, W., and Hasselblatt, A. (1970) Arch. Pharmakol., 266, 420-421.
17. Montague, W. and Taylor, K. W. (1970) The Structure and Metabolism of the Pancreatic Islets, A Centennial of Paul Langerhans' Discovery, pp. 263-273, Pergamon Press, Oxford.
18. Ammon, H. P. T. (1975) Arch. Pharmakol., 290, 251-264.
19. Panten, U., and Christians, J. (1973) Arch. Pharmakol., 276, 55-62.
20. Ammon, H. P. T., Patel, T. N., and Steinke, J. (1973) Biochim. Biophys. Acta, 297, 352-367.
21. Hellman, B., and Taljedal, I-B. (1970) J. Cell Biol. 47, 289-293.
22. Ammon, H. P. T., and Steinke, J. (1972) Diabetes, 21, 143-148.
23. Deery, D. J., and Taylor, K. W. (1973) Biochem. J., 134, 557-563.
24. Bloom, G. D., Hellman, B., Idahl, L-A., Lernmark, A., Sehlin, J., and Taljedal, I-B. (1972)Biochem. J., 129, 241-254.
25. Jocelyn, P. C. (1972) Biochemistry of the SH Group, pp. 74, 190, Academic Press, New York.
26. Wins, P. (1969) Arch. Int. Physiol. Biochim., 77, 251-259.