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EFFECT OF ETHANOL AND *n*-BUTANOL ON ALLOXAN INHIBITION OF GLUCOSE-INDUCED INSULIN RELEASE IN ISOLATED PANCREATIC ISLETS*

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Abstract—The aliphatic alcohols, ethanol and *n*-butanol, were evaluated as protective agents against alloxan inhibition of insulin release in isolated rat pancreatic islets. Ethanol and *n*-butanol were ineffective in preventing alloxan inhibition of glucose-induced insulin release when present during the alloxan exposure, as well as during pretreatment conditions.

The aliphatic alcohols, methanol, ethanol, *n*-propanol and *n*-butanol, have been reported to prevent the diabetogenic action of alloxan in mice [1, 2]. These alcohols were shown to react with free hydroxyl radicals (·OH) produced *in vitro* in an ethylene-generating system. The order of reactivity of these alcohols with the hydroxyl radical was found to parallel, reasonably well, their ability to provide protection against alloxan. Since the autooxidation of dialuric acid, the reduction product of alloxan, would result in the formation of this cytotoxic species (·OH), a possible role for the hydroxyl radical in the diabetogenic action of alloxan has been proposed [1].

Recent studies *in vitro* in a static incubation system have demonstrated that exposure of isolated rat pancreatic islets to alloxan for 5 min inhibits subsequent glucose-induced insulin secretion [3]. The presence of either D-glucose, D-mannose, or 3-*O*-methyl-D-glucose during this brief alloxan exposure provides almost complete protection of the beta cells from the inhibitory effect of alloxan. The protection provided by D-glucose against alloxan inhibition of insulin release exhibits anomer preference with the alpha anomer of D-glucose, providing more protection than the beta anomer [4]. These findings suggest that D-glucose and alloxan may interact at a common receptor-site involved in the insulin release mechanism of the beta cell.

The purpose of the present investigation was to employ this system *in vitro* to evaluate the ability of the aliphatic alcohols, ethanol and *n*-butanol, to prevent alloxan inhibition of glucose-induced insulin release in isolated pancreatic islets of Langerhans.

MATERIALS AND METHODS

Isolated pancreatic islets were obtained by collagenase digestion [5, 6] from adult (200–300 g) male Wistar rats allowed food and water *ad lib*. Static incubations were performed in a Krebs–Ringer bicarbonate (KRB) medium containing 115 mM NaCl, 1.0 mM $MgCl_2$, 24 mM $NaHCO_3$, 5.0 mM KCl, 2.5 mM $CaCl_2$, and albumin, 0.5% (w/v), Armour Pharmaceutical Co., Chicago, IL. The medium was equilibrated to pH 7.4 with a mixture of humidified O_2/CO_2 (95%/5%).

Alloxan monohydrate was obtained from Sigma Chemical Co., St. Louis, MO; D-glucose (Dextrose) from the National Bureau of Standards, Washington, DC; ethanol from U.S. Industrial Chemicals Co., New York, NY; and *n*-butanol from Mallinckrodt Co., St. Louis, MO.

After isolation, pancreatic islets were transferred with the aid of a glass loop and dissection microscope to round-bottomed vials (11 mm dia × 20 mm height) which contained 200 μl of KRB medium supplemented with D-glucose (5.5 mM). Each vial contained twenty to twenty-five islets, and twelve to fifteen vials were employed per experiment. The vials were placed in scintillation vials equipped with rubber stoppers for subsequent gassing and maintained at 37°C by a Dubnoff metabolic shaker (100 strokes/min) water bath. Removal and addition of medium (200 μl) were accomplished with the aid of a constriction pipette.

The basic protocol consisted of a preincubation of 30 min performed in a glucose-free medium. After this preincubation period, the medium was removed and replaced with medium containing either alloxan alone or alloxan in the presence of the test agent for a subsequent 5-min exposure. The medium was then removed and replaced with medium containing D-glucose (27.5 mM) for a 30-min insulin stimulation period. In pretreatment experiments, the same basic protocol was employed with the exception that the alcohol was present during the 30-min preincubation in addition to the 5-min alloxan exposure.

Insulin release from islets exposed to alloxan alone or alloxan in the presence of the test agent was compared to that of a parallel group of islets in which

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the alloxan exposure was omitted. At the termination of the stimulation period, the medium was removed and frozen for subsequent insulin assay by the method of Wright *et al.* [7]. Porcine insulin was used as a standard with ^{125}I-labeled porcine insulin obtained from New England Nuclear, Boston, MA, as tracer. Results are expressed as μU insulin/islet/min with the data presented as the mean ± S.E.

Alloxan assay. A stock solution of alloxan was prepared in 10^{-3} N HCl. The alloxan concentration was determined at 270 nM ($E = 980\ M^{-1}\ cm^{-1}$) in 50 mM phosphate buffer, pH 7.4, by the method of Patterson *et al.* [8]. Aliquots of the stock solution (54 mM) were added to previously warmed and gassed medium 10 sec prior to use.

To determine whether a chemical interaction occurred between alloxan and the test agents, the decomposition rate of alloxan (650 μM) was monitored by the 1,2-phenylenediamine assay of Belic and Felber [9]. Samples (4–20 μl) of the Krebs–Ringer medium at pH 7.4 and 37° containing alloxan in the absence and presence of the alcohols were removed at 0.5-min intervals. The amount of product formed between alloxan and 1,2-phenylenediamine was measured fluorometrically, and the medium alloxan concentration calculated by comparison with the fluorescence produced from alloxan standards incubated in the 1,2-phenylenediamine assay system. In these measurements, the $t½$ decomposition rate for alloxan (650 μM) was 1.10 min. In the presence of ethanol (100 mM) and *n*-butanol (100 mM), the $t½$ decomposition rates for alloxan were 1.14 and 1.04 min respectively. The presence of ethanol and *n*-butanol in Krebs–Ringer medium had no detectable effect on the decomposition rate of alloxan.

Alloxan exposure. In previous studies, the effect of different alloxan concentrations on glucose-induced insulin release was determined in a static incubation system [3]. A 5-min exposure of islets to alloxan (650 μM) was the minimal time vs concentration required to produce maximal inhibition (94 per cent) of glucose-induced insulin release. The presence of D-glucose (27.5 mM) during the 5-min alloxan exposure protected against the inhibitory effect of alloxan and resulted in a rate of insulin release 88 per cent of that observed in non-alloxan-treated islets.

RESULTS

In evaluating the ability of the aliphatic alcohols, ethanol and *n*-butanol, to prevent alloxan inhibition of insulin release, alcohol concentrations were used comparable to those employed in the studies *in vivo* reported by Heikkila *et al.* [1, 2]. In initial experiments, the direct effect of alcohol exposure on subsequent insulin release by pancreatic islets was determined. After preincubation in a glucose-free medium for 30 min, islets were exposed to either ethanol (100 mM) or *n*-butanol (100 mM) for a pulse period of 5 min. The rate of insulin release from these islets was then determined over a subsequent 30-min incubation period in the presence of D-glucose (27.5 mM). The rate of insulin release from untreated control islets was 3.81 ± 0.26 μU/islet/min compared with rates of 3.42 ± 0.28 and 3.29 ± 0.33 from islets exposed to ethanol and *n*-butanol respectively (N = 8 at each experimental condition). These results indicate that alcohol exposure for 5 min prior to stimulation did not alter subsequent glucose-induced insulin release.

In the evaluation of ethanol and *n*-butanol as protective agents against alloxan inhibition of insulin release, this same protocol was employed. As indicated in Tables 1 and 2, neither the presence of ethanol nor that of *n*-butanol at 50, 75 or 100 mM during the 5-min alloxan exposure prevented the inhibitory effect exerted by alloxan on subsequent glucose-induced insulin release. No differences were observed in the rates of insulin release by islets exposed to alloxan in the absence or presence of these aliphatic alcohols.

In order to maximize islet exposure to the test alcohols, the effect of pretreatment of islets with either ethanol or *n*-butanol on alloxan inhibition of insulin release was also evaluated. As indicated in Table 3 (part A), pretreatment of islets for 30 min with ethanol (100 mM) prior to stimulation did not affect subsequent glucose-induced insulin release. In alloxan-treated islets, the presence of ethanol during the 30-min preincubation and alloxan exposure did not prevent inhibition of subsequent glucose-induced insulin release (Table 3, part B).

Pretreatment conditions with *n*-butanol present in the incubation medium for 30 min indicated that a

Table 1. Effect of ethanol pulse on alloxan exposure of isolated islets*

Experimental conditions (5-min exposure)	Insulin release (μU/islet/min)		
	Alcohol (50 mM)	Alcohol (75 mM)	Alcohol (100 mM)
Control	2.41 ± 0.36 (8)	2.05 ± 0.15 (8)	5.75 ± 0.44 (12)
Ethanol + alloxan (650 μM)	0.54 ± 0.15 (8)	0.25 ± 0.10 (8)	0.77 ± 0.15 (12)
Alloxan (650 μM)	0.58 ± 0.15 (8)	0.21 ± 0.06 (8)	0.61 ± 0.09 (12)

* After a 30-min preincubation in a glucose-free medium, islets were exposed to alloxan in the absence and presence of ethanol for 5 min, or the exposure period was omitted (control). The medium was removed, and replaced with medium containing D-glucose (27.5 mM) for a 30-min stimulation period. Results are expressed as the mean ± S.E. with the number of observations in parentheses.

Table 2. Effect of *n*-butanol pulse on alloxan exposure of islated islets*

Experimental conditions (5-min exposure)	Insulin release (μU/islet/min)		
	Alcohol (50 mM)	Alcohol (75 mM)	Alcohol (100 mM)
Control	2.76 ± 0.22 (8)	3.33 ± 0.30 (8)	3.24 ± 0.27 (12)
n-Butanol + alloxan (650 μM)	0.33 ± 0.06 (8)	0.29 ± 0.06 (8)	0.94 ± 0.12 (12)
Alloxan (650 μM)	0.46 ± 0.05 (8)	0.35 ± 0.10 (8)	0.71 ± 0.15 (12)

* Experimental conditions were identical to those described in Table 1.

Table 3. Effect of pretreatment with alcohols on alloxan exposure of isolated islets*

Experimental conditions (pretreatment period)	Insulin (μU/islet/min)
(A) Control	3.54 ± 0.09 (8)
Ethanol (100 mM)	3.53 ± 0.16 (8)
(B) Control	3.52 ± 0.17 (12)
Ethanol (100 mM)	0.42 ± 0.06 (12)
Alloxan (650 μM)	
(C) Control	3.11 ± 0.16 (12)
n-Butanol (10 mM)	2.71 ± 0.17 (8)
n-Butanol (10 mM)	0.32 ± 0.07 (12)
Alloxan (650 μM)	

* Incubation conditions were identical to those described in Table 1. (A) Islets were preincubated with ethanol for 30 min or the ethanol preincubation was omitted (control). (B) Islets were exposed to alloxan and ethanol for 5 min after a 30-min preincubation with ethanol, or only the alloxan exposure was omitted (control). (C) Islets were preincubated for 30 min in the presence and absence of *n*-butanol (control) or exposed to alloxan and *n*-butanol for 5 min after a 30-min preincubation with *n*-butanol.

concentration of 10 mM did not inhibit subsequent glucose-induced insulin release. An *n*-butanol concentration of 100 mM as used in the previous 5-min pulse protocol, however, was inhibitory under these pretreatment conditions. In alloxan-treated islets, the presence of *n*-butanol (10 mM) during the preincubation and alloxan pulse period did not alter the inhibitory effect exerted by alloxan on glucose-induced insulin release (Table 3, part C).

DISCUSSION

In the present study, ethanol and *n*-butanol were evaluated as protective agents against alloxan inhibition of insulin release in isolated rat pancreatic islets. Ethanol and *n*-butanol were ineffective in preventing alloxan inhibition of glucose-induced insulin release. In this experimental protocol, the alcohols were evaluated when present during alloxan exposure, as well as during pretreatment conditions. The alcohol concentrations and preincubation periods in these studies *in vitro* represented conditions optimal for alcohol exposure without exerting an inhibitory effect on glucose-induced insulin release.

Previous studies *in vivo* [1] have indicated that the aliphatic alcohols, methanol, ethanol, *n*-propanol and *n*-butanol, prevented alloxan-induced hyperglycemia in mice. It was postulated that hydrogen peroxide (H_2O_2), the superoxide radical (O_2^-) or the hydroxyl radical ($^{\cdot}OH$) may be involved in the diabetogenic action of alloxan. In this postulated mechanism, dialuric acid, the reduction product of alloxan, upon auto-oxidation by oxygen results in the formation of these reactive species [1]. The protection *in vivo* reported by Heikkila *et al.* [1] against alloxan toxicity by the aliphatic alcohols was consistent with a role for the hydroxyl radical and inconsistent with roles for either hydrogen peroxide or the superoxide radical in the diabetogenic action of alloxan.

The present system *in vitro* permits direct measurement of the effect of alloxan on insulin release from pancreatic islets. Since ethanol and *n*-butanol had no protective effect against alloxan, this study provides no evidence that alloxan inhibition of insulin release, *in vitro*, is mediated by a free-radical mechanism. Based on the present data, prevention of the diabetogenic action of alloxan by aliphatic alcohols may involve parameters other than alloxan's direct interaction with the insulin release mechanism.

REFERENCES

1. R. E. Heikkila, B. Winston, G. Cohen and H. Barden, *Biochem. Pharmac.* **25,** 1085 (1976).
2. R. E. Heikkila, H. Barden and G. Cohen, *J. Pharmac. exp. Ther.* **190,** 501 (1974).
3. D. C. Weaver, M. L. McDaniel and P. E. Lacy, *Diabetes* (Abstr.) **26,** (suppl. 1), 380 (1977).
4. M. L. McDaniel, C. E. Roth, C. J. Fink and P. E. Lacy, *Endocrinology* **99,** 535 (1976).
5. P. E. Lacy and M. Kostianovsky, *Diabetes* **16,** 35 (1967).
6. P. E. Lacy, M. M. Walker and C. J. Fink, *Diabetes* **21,** 987 (1972).
7. P. H. Wright, D. K. Maksalu, D. Virchick and K. E. Sussman, *Diabetes* **20,** 33 (1971).
8. J. W. Patterson, A. Lazarow and S. Levy, *J. biol. Chem.* **177,** 187 (1949).
9. N. Belic and J. P. Felber, *Analyt. Biochem.* **29,** 91 (1969).