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Effects of Anti-Microtubular Agents on Alloxan Stimulation and Inhibition of Glucose-Induced Insulin Release in Vitro*)

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ABSTRACT

The effect of various anti-microtubular agents on alloxan stimulation and inhibition of a subsequent glucose-induced insulin release was investigated using in vitro incubation and perifusion of rat isolated islets of Langerhans. Five minute exposure of islets to alloxan (20 mg/dl) induced a transient burst of insulin release which was eliminated by the pretreatment of islets by heavy water (D_2O) or colchicine (10^{-3} M) and concomitant presence of them with alloxan. However, vincristine (10^{-4} M) did not affect alloxan actions.

A brief exposure of islets to alloxan completely inhibited a subsequent glucose (16.7 mM)-induced insulin release. The pretreatment of islets with D_2O or colchicine prevented alloxan inhibition of insulin release, whereas vincristine did not demonstrate such an action.

Because the transport of pyrimidine was completely inhibited by anti-microtubular agents, it seems likely that the transport of alloxan (2, 4, 5, 6-tetraoxypyrimidine, 5, 6-dioxyuracil) into the B-cell is impaired by these agents. Otherwise, anti-microtubular agents reduced the generation of highly reactive oxygen-containing free radicals from alloxan, which inhibits the glucose-induced insulin release.

INTRODUCTION

Alloxan has been reported to inhibit glucose-induced insulin release from the isolated islets of Langerhans[5,21]. The α-anomer of D-glucose which is more effective than the β-anomer in stimulating insulin release[3,16], is also preferential for elimination of alloxan inhibition of glucose-induced insulin release[13,17]. It has been postulated that alloxan interacts with a hypothetical glucose receptor resulting in competitive inhibition with the glucose molecule[17].

It is well known that various effectors of insulin secretion have protective effects against alloxan action[10,12]. We already reported that cytochalasin B, microfilament-disrupting agent, completely prevented alloxan inhibition of glucose-induced insulin release[5]. However, the effects of anti-microtubular agents on alloxan action have never been srudied.

It has been reported that alloxan itself induced a burst of insulin release from perifused islets[24] and from the perfused rat isolated pancreas[19], which have been considered as an evidence for alloxan action on hypothetical glucose receptor.

In the present study, we investigate the effects of various anti-microtubular agents on alloxan stimulation and inhibition of subsequent glucose-induced insulin release in the perifused rat isolated islets. We discuss the protection mechanism of anti-microtubular agents against alloxan action.

MATERIALS AND METHODS

All experiments were performed with isolated islets from fed male Wistar albino rats (300-

*) 石橋不可止：Anti-microtubular agent のアロキサンによるインスリン分泌およびブドウ糖によるインスリン分泌阻害に及ぼす影響

350 g). The perifusion system used here has been reported[5,6]. Briefly, a double chamber technique was employed. Two identical chambers were perifused simultaneouly by the same peristaltic pump with one chamber serving as a control. The basal perifusate consists of Krebs-Ringer bicarbonate (KRB) medium containing bovine serum albumin, 5 mg/ml ; NaCl, 140mM ; KCl, 5 mM ; KH_2PO_4, 1 mM ; $CaCl_2$, 2 mM ; $MgCl_2$, 1 mM and $NaHCO_3$, 20 mM. The medium was kept at 37°C and continuously gassed with 95% O_2 and 5% CO_2 maintaining the pH at 7.35–7.45. The flow rate was adjusted to 1.6 ml/min.

Isolated islets were preincubated for 60 minutes with basal medium in the presence or absence of anti-microtubular agents at 37°C. The pH of medium was kept at 7.4. Then, each of fifteen islets was placed in the perifusion chembers and preperifused for 30 minutes by basal medium with or without anti-microtubular agents. This was followed by a 5 minute perifusion with alloxan (20 mg/dl) with or without antimicrotubular agents (10^{-3} M colchicine or 10^{-4} M vincristine). In experiment with D_2O, medium was made of pure D_2O with the same ionic compositions. The third perifusion used the basal medium for 10 minutes followed by 16.7 mM glucose for 40 minutes.

Insulin content in perifusates was determined by the double antibody radioimmunoassay method of Morgan and Lazarow[15].

A half life of alloxan in phosphate buffer (pH 7.4) at 37°C determined spectrophotometrically[20] was 90 seconds. The coexistence of anti-microtubular agents with alloxan did not modify the decomposition rate of alloxan.

The materials employed and their sources were as follows; Collagenase type IV 160 U/mg (Worthington Biochem. Co.), bovine serum albumin, D-glucose and alloxan monohydrate (Sigma Chem. Co.), colchicine (Merk Co.), D_2O (CEA, France) and ^{125}I-insulin (Radiochemical Center, Engl.).

Values are mean±SEM, and analyzed by unpaired t-tests.

RESULTS

Fig. 1 illustrates alloxan stimulation and inhibition of subsequent glucose-induced insulin release. Five minute exposure of islets to alloxan induced a burst of insulin release (peak value; 2.30±0.34 μU/islet/min) and inhibited completely glucose-induced biphasic insulin release (Table).

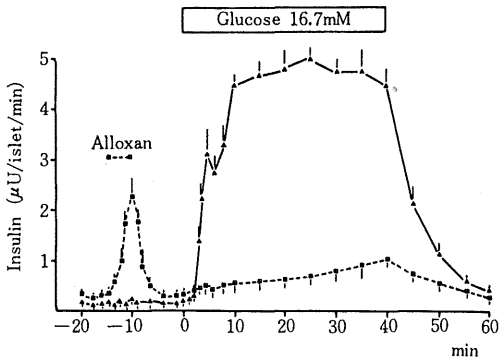

Fig. 1. Alloxan stimulation and inhibition of subsequent glucose-induced insulin release. After incubation for 60 minutes with basal medium, fifteen islets were preperifused for 30 minutes with basal medium and exposed to alloxan (20 mg/dl) for 5 minutes (■·····■). The control chamber was perifused with basal medium (▲——▲). After washing for 10 minutes, islets were exposed to 16.7 mM glucose for 40 minutes. Insulin contents in perifusates were determined by a double antibody radioimmunoassay method.
Values are mean±SEM of 8 perifusions.

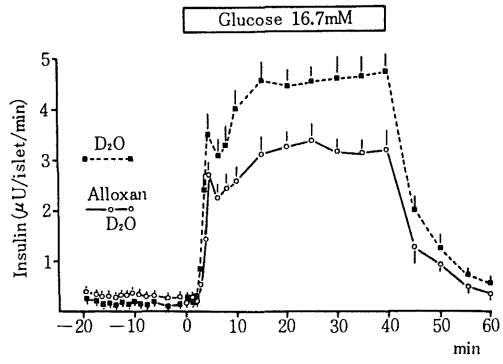

Fig. 2. Effects of heavy water (D_2O) on alloxan stimulation and inhibition of insulin release. Islets were incubated by basal medium made from pure D_2O for 60 minutes at 37°C. Then, islets were transferred into the perifusion chamber and preperifused for 30 minutes with the same medium followed by an exposure of islets to alloxan (20 mg/dl) dissolved in the same medium for 5 minutes. After washing for 10 minutes with basal medium, 16.7 mM glucose was perifused for 40 minutes.
Values are mean±SEM of 5 perifusions.

Fig. 2 reveals the effect of D_2O on alloxan stimulation and inhibition of subsequent glu-

Table Protection by various anti-microtubular agents against alloxan inhibition of glucose-induced insulin release. * Islets were preincubated for 60 minutes with vincristine (10^{-4} M) and transferred into the perifusion chamber. After preperifusion for 30 minutes with the same medium, islets were exposed to alloxan (20 mg/dl) for 5 minutes. These islets were washed for 10 minutes followed by a perifusion with 16.7 mM glucose for 40 minutes (n=5). Otherwise, the methods of experiments were stated in the legends of pertinent figures.

5 MIN PERIFUSION	INSULIN, MEAN±SEM (μU/islet/40 min)	% OF NON-ALLOXAN TREATED CONTROL	P Value
Glucose 2.7 mM	164.5±13.2		
+Alloxan 20 mg/dl	30.4± 4.2	18.4±2.6	—
D_2O	157.4±11.3		
+Alloxan 20 mg/dl	110.8±10.5	70.4±6.7	$p<0.001$
Colchicine 10^{-3} M	156.4±14.5		
+Alloxan 20 mg/dl	109.0±10.4	69.7±6.6	$p<0.001$
Vincristine 10^{-4} M*	153.4±15.8		
+Alloxan 20 mg/dl	27.4± 3.8	17.9±2.5	N.S.

P; compared with value in alloxan treated islets.

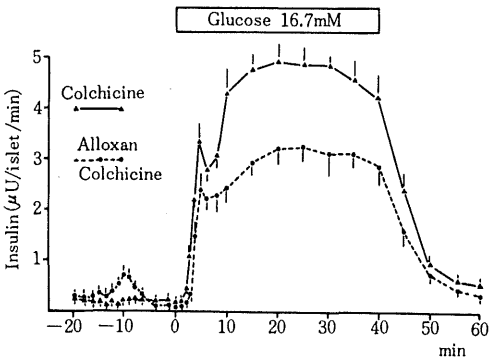

Fig. 3. Effects of colchicine on alloxan stimulation and inhibition of insulin release. Islets were incubated for 60 minutes in basal medium containing colchicine (10^{-3} M), placed in the perifusion chamber and preperifused for 30 mitutes with the same medium. After exposure to alloxan (20 mg/dl) for 5 minutes, islets were washed for 10 minutes and exposed to 16.7 mM glucose for 40 minutes. Values are mean±SEM of 6 perifusions.

cose-induced insulin release. The pretreatment of the islets by D_2O and its concomitant presence with alloxan totally abolished alloxan-induced insulin release (peak value; 0.31±0.03 μU/islet/min) and protected the islets against alloxan inhibition of glucose-induced insulin release (Table). The mean values for the first and the second peak of 2.70±0.16 μU/islet/min and 3.35±0.29 μU/islet/min, respectively, were significantly higher than corresponding values found in the islets exposed to alloxan in water (Fig. 1).

As shown in Fig. 3 the pretreatment of islets by colchicine and its simultaneous presence with alloxan provided a significant protection of the islets against alloxan inhibition of glucose-induced insulin release. Colchicine also significantly suppressed the alloxan-induced burst of insulin release (peak value; 0.74±0.14 μU/islet/min).

On the other hand, vincristine did not protect islets against alloxan inhibition, nor modify the alloxan-induced insulin release (Table, Figure not shown).

DISCUSSION

The present study revealed that D_2O and colchicine abolished alloxan-induced insulin release, whereas the alloxan inhibition of glucose-induced insulin release was prevented by the pretreatment and the concomitant presence of these agents with alloxan.

Alloxan induces a transient burst of insulin release[24], and then inhibits a subsequent glucose-induced insulin release[5,21] with the capability to induce insulin release when stimulated by secretagogues other than glucose[23]. Since the α-anomer of D-glucose[13,17] or 3-0-methylglucose[21] eliminated alloxan inhibition of glucose-induced insulin release, alloxan has been considered to interact with glucose at the site where D-glucose initiates insulin release in the pancreatic B-cells. The mechanisms of alloxan action, however, remained to be elucidated. The understanding of protection mechanisms of agents other than glucose against alloxan may clarify the action mechanism of alloxan in pancreatic B-cells.

It has been known that the uptake of pyri-

midine occurs by a facilitated diffusion, and alloxan, 2, 4, 5, 6-tetraoxy-pyrimidine, 5, 6-dioxyuracil is incorporated into isolated islets by facilitated diffusion[25]. Anti-microtubular agents inhibit pyrimidine transport, whose action appeared to be unrelated to their action on microtubules[14]. This becomes more relevant, since the transport related agents cytochalasin B[5,12], phloridin[22] and phosphodiesterase inhibitors[10] also protected pancreatic B-cells against alloxan inhibition of insulin release in vitro. However, antimicrotubular agents could not impair glucose and adenosine nucleotide metabolism in the islet[11]. Recently, it was postulated that β-cytotoxic action or the inhibition of glucose-induced insulin release by alloxan is mediated by the generation of highly reactive oxygen-containing free radicals from alloxan[4], and the inhibition of generation of these radicals by catalase, superoxide dismutase or ion scavenger abolished alloxan inhibition of glucose-induced insulin release in incubated islets of Langerhans[2]. In the previous report we found that alloxan had a biphasic action on glucose metaboilsm in cultured fibroblasts-transient initial stimulation and subsequent inhibition[7,8]. The biphasic action on glucose metabolism was caused by generation of oxygen-containing free radicals, since the inhibition of the generation of these radicals resulted in the prevention of alloxan action on glucose metabolism[9]. Anti-microtubular agents modified the generation of superoxide anion and activity of NAD(P)H oxidase in polymorphonuclear leukocytes[18]. The presence of superoxide dismutase was confirmed immunocytochemically[1] and alloxan reduced the activity of superoxide dismutase in the pancreatic islets[1]. These observations might suggest that alloxan and anti-microtubular agents interact at the generation system of oxygen-containing free radicals in the pancreatic B-cells. However, we can not explain the failure of vincristine to prevent alloxan action.

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