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Effects of alloxan on electric activity of mouse pancreatic B cells *in vitro*

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ABSTRACT Microelectrode method for recording membrane potentials was used. It was observed that alloxan possessed stimulative and toxic dual effects on the electric activity of pancreatic B cells. A 10-min exposure to alloxan 14 mmol·L^{-1} in the perfusion medium without glucose caused a significant depolarization of B cells from -44 ± 13 mV to -36 ± 12 mV ($n=7$, $P<0.05$) and evoked spikes in B cells. But the spikes disappeared at 24 ± 12 min ($n=8$). In the presence of glucose 5.5 mmol·L^{-1} or 11.0 mmol·L^{-1}, the dual effects became weaker or not obvious. After 10-min exposure to diazoxide (an ATP sensitive K^+ channel opener) 0.8 mmol·L^{-1} in the perfusion medium with alloxan 14 mmol·L^{-1} but devoid of glucose, the amplitude of membrane potential went up from -31 ± 11 mV to -48 ± 21 mV and the mean value of spikes dropped from 31 ± 24 spikes·min^{-1} to 5 ± 5 spikes·min^{-1}. It was suggested that the stimulative effect of alloxan was due to blocking of ATP sensitive K^+ channel and a higher concentration of glucose could prevent B cells from alloxan injury.

KEY WORDS membrane potentials; islets of Langerhans; alloxan; diazoxide

Alloxan has been used for decades to induce diabetes mellitus in experimental animal because of its selective toxic effect on the insulin-producing pancreatic B cells. A hypothesis has been proposed that the chronic toxicity of alloxan to B cells resulted in alloxan-induced break of DNA strand by hydroxyl radical[1]. It explains the chronic hyperglycemia, but not the initial acute severe hypoglycemia. It was confirmed that the electric activity of B cells coupled with insulin secretion[2]. It has been shown that glucose depolarized pancreatic B cells by closing K^+ channel which is regulated by intracellular ATP[3,4]. Diazoxide, a kind of ATP sensitive K^+ (ATP-K^+) channel openers, can abolish glucose-stimulated insulin release, increase K^+ efflux, and inhibit glucose-induced electric activities of the B cell[5]. It is necessary to investigate whether the hypoglycaemic effect of alloxan are correlated to the closure of ATP-K^+ channel or not.

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MATERIALS AND METHODS

The intracellular electrode recording of membrane potentials on mouse pancreatic islet cells was the same as that described previously[6]. The perfusion fluid was modified mouse Krebs solution (NaCl 110, $NaHCO_3$ 25, KCl 5, $CaCl_2$ 2.56, and $MgCl_2$ 1.13 mmol·L^{-1}) gassed with 95 % O_2 + 5 % CO_2, pH 7.4. Alloxan and diazoxide were phurchased from Sigma. All values were expressed as $\bar{x}\pm s$. Data were analyzed by paired t test.

RESULTS

Effect of alloxan on electric activity of B cells in different concentrations of glucose

The resting membrane potentials of B cells in the absence of glucose showed -44 ± 13 mV ($n=7$) and no spike appeared (Fig 1A). After the addition of alloxan 3.5 mmol·L^{-1}, the islet B cells began to be depolarized, but no spike was seen. The spikes were induced only after alloxan 7, 14, 28, and 56 mmol·L^{-1}

were added. The spike frequencies within 20 min after alloxan were 7.1±2.3 (n=6), 14.7 ±5.1 (n=6), 12.6±2.4 (n=6), 13.4±3.0 (n=6) spikes·min^{-1} successively. After a 10-min exposure to alloxan 14 mmol·L^{-1} the B cells depolarized to −36±12 mV (n=7, P < 0.05 *vs* before alloxan) (Fig 1E). The spikes were observed at about 10 min (Fig 1B) and then disappeared at 24±12 min (n=8) (Fig 1C). Sixty minutes later, alloxan was replaced by glucose 5.5 mmol·L^{-1} (suprathreshold concentration for activation of B cells), but no spike was found during the period of 30-min exposure to glucose (Fig 1D, Fig 2A).

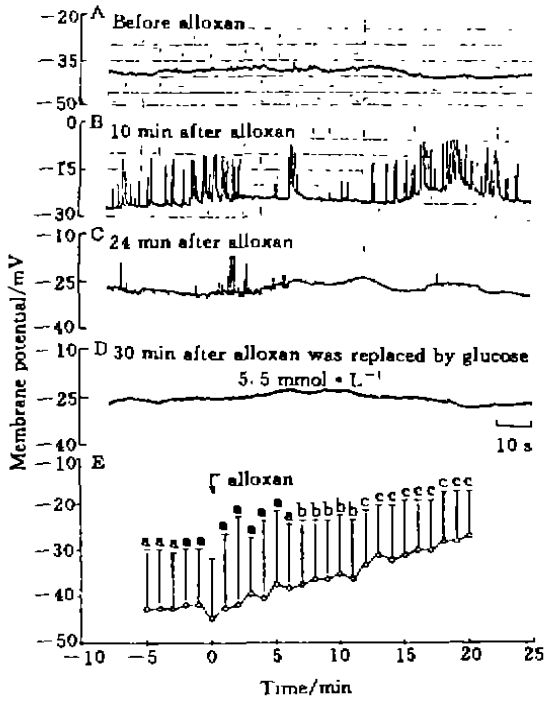

Fig 1. Effect of alloxan 14 mmol·L^{-1} on membrane potentials of B cells in the absence of glucose. Records A to D from the same cell. E, n= 7 cells, $\bar{x}$ ±s. ^aP>0.05, ^bP<0.05, ^cP<0.01 *vs* before alloxan was added.

In the presence of glucose 2.75 mmol·L^{-1} the resting membrane potentials of B cells were −36±12 mV (n=6) and no spike was shown. After the addition of alloxan 14 mmol·L^{-1}, the cells were depolarized further. After a 10-min exposure, the B cells depolarized to −26±10 mV (n=6, P<0.01 *vs* before alloxan) and the spikes emerged. After 60 min, alloxan was replaced by glucose 5.5 mmol·L^{-1} and no spike was seen during the 30-min exposure to glucose (Fig 2B).

The spike frequency of B cells in the presence of glucose 11.0 mmol·L^{-1} was higher than that in 5.5 mmol·L^{-1}. After the addition of alloxan 14 mmol·L^{-1}, the spike frequency also increased in the presence of glucose 5.5 mmol·L^{-1}, but the spikes disappeared after 48±12 min (n=6). After 60 min, alloxan was washed out, and the spikes reappeared (Fig 2C). When the glucose concentration was 11.0 mmol·L^{-1}, the excitatory effect of alloxan 14 mmol·L^{-1} on B cells was not obvious, and the depressant effect was not complete. After alloxan was washed out, the spike frequency increased again (Fig 2D).

Effect of diazoxide on excitatory activity of B cells induced by alloxan The electric activity of B cells in the presence of glucose 5.5 mmol·L^{-1} was inhibited by diazoxide 0.2, 0.4, 0.8, 1.6, and 3.2 mmol·L^{-1}. The inhibitory rates of spikes within 10 min after diazoxide were 20±3% (n=6), 40±7% (n=6), 64±5% (n=6), 64±4% (n=6), 66±5% (n=6) in succession. After the excitatory effect of B cells induced by alloxan 14 mmol·L^{-1} in the absence of glucose was observed, diazoxide 0.8 mmol·L^{-1} was added to the medium. After 10 min, the membrane potentials went up from −31±11 mV to −48±21 mV (n=6, P<0.05) (Fig 3A, 3B) and the spike frequency dropped from 31±24 spikes·min^{-1} to 5±5 spikes·min^{-1} (n=6, P<0.05) (Fig 3E). Ten minutes after diazoxide was washed out, the membrane potentials decreased to −35±11 mV again (n=6,

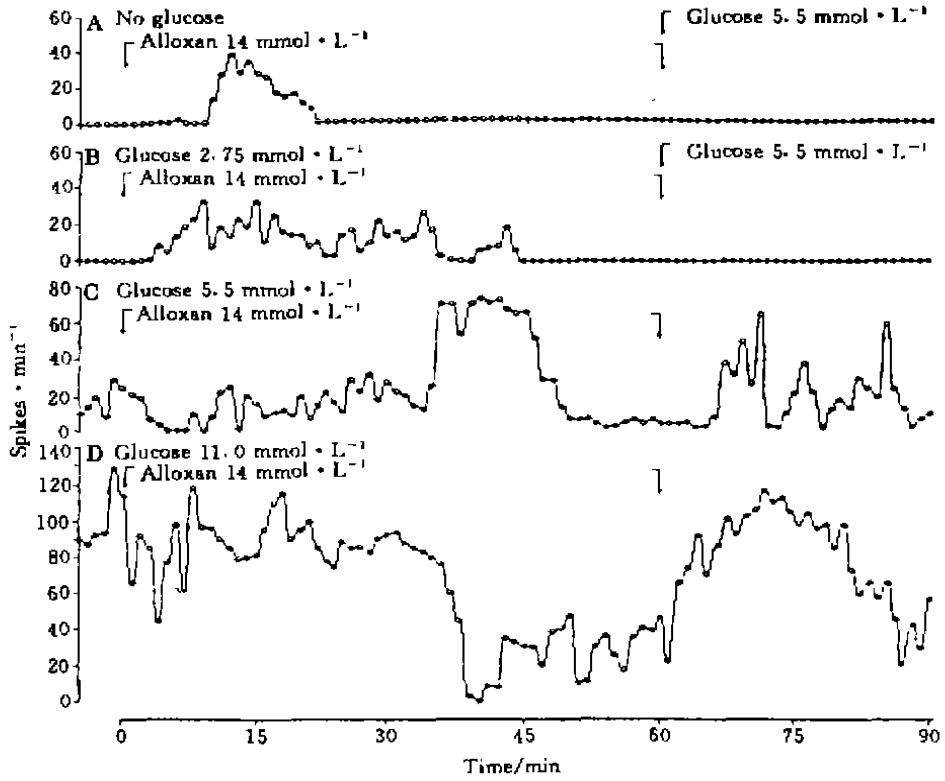

Fig 2. Effect of alloxan on spikes of B cells in presence of glucose.

$P>0.05$ *vs* before diazoxide) and the spike frequency increased to 11 ± 5 spikes $\cdot$ min^{-1} ($n=6$, $P>0.05$ *vs* before diazoxide) (Fig 3E).

DISCUSSION

The present results demonstrated that alloxan was able to cause depolarization of pancreatic B cells, which was similar to the findings of Dean and Mathews[7]. Furthermore, we observed the discharge of B cells induced by alloxan. ATP-K^+ channel opener diazoxide caused the hyperpolarization of B cells and blocked the discharge of B cells induced by alloxan. It revealed that alloxan, like glucose, could induce the discharge of B cells through blocking ATP-K^+ channel. The results supported the report of Kozlowski and Ashford (1991) in that alloxan inhibited ATP-K^+ channel currents in outside-out membrane patches[8]. It could explain the initial acute severe hypoglycemia in the experimental diabetes induced by alloxan[9].

It was observed that the actions of alloxan were not the same in different concentration of glucose. Without glucose or with glucose 2.75 mmol $\cdot$ L^{-1}, alloxan could cause B cells to discharge. After a period of continuous discharging, the spikes disappeared. After the alloxan was removed, supra-threshold concentration of glucose was unable to reverse its effect. This revealed that alloxan possessed both stimulative and toxic dual effects. In the presence of glucose 5.5 mmol $\cdot$ L^{-1}, the discharging time of spikes was longer than that in the absence of glucose significantly

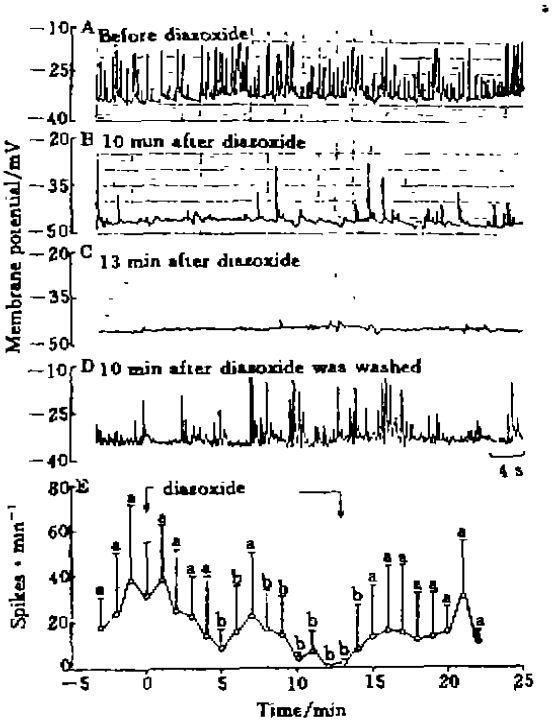

Fig 3. Effect of diazoxide 0.8 mmol·L^{-1} on the membrane potentials and spikes of B cells in the presence of alloxan 14 mmol·L^{-1} but in the absence of glucose. Records A to D from the same cell. E: $n=6$ cells $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$ *vs* before diazoxide was added.

($P<0.01$). After removal of alloxan, B cells was still able to discharge again. In the glucose 11.0 mmol·L^{-1}, the depressant effect of alloxan was attenuated. It was suggested that higher concentration of glucose protected B cells from the toxic action of alloxan.

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15

四氧嘧啶对离体小鼠胰岛 B 细胞电活动的影响

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摘要 记录细胞内电位发现四氧嘧啶具有激活和毒化胰岛 B 细胞的双相作用. 在无或2.75 mmol·L^{-1}葡萄糖时四氧嘧啶使 B 细胞膜去极化并诱发短暂的能被 ATP 敏感钾通道开放剂二氮嗪阻断的放电；在葡萄糖5.5或11.0 mmol·L^{-1}时作用减轻或不明显. 提示四氧嘧啶的激活作用与关闭 ATP 敏感的钾通道有关，而高葡萄糖能保护 B 细胞免受四氧嘧啶的损伤.

关键词 膜电位；胰岛；四氧嘧啶；二氮嗪