

Effect of ATP-Dependent K⁺ Channel Openers and Blockers on Serum Concentration of Aldosterone in Rats

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ABSTRACT

There are many reports for involvement of ATP-sensitive potassium channels in pancreatic, cardiac and vascular smooth muscle cells. This study examined the effect of single doses of K⁺ channel openers; diazoxide, minoxidil and K⁺ channel blockers; chlorpropamide, glibenclamide on serum concentration of aldosterone in male rats. Blood samples were obtained 60 minutes after drug treatment and serum aldosterone level was determined by RIA. The basal serum aldosterone was 659.32 ± 71.48 pg/ml and after diazoxide or minoxidil administration increased to 1188.53 ± 99.45 pg/ml and 1392.69 ± 177.83 pg/ml, respectively. Chlorpropamide or glibenclamide treatment did not produce any change in basal serum aldosterone concentration, but in early streptozotocin-induced diabetic rats decreased serum aldosterone level significantly ($P < 0.001$). Pretreatment with glibenclamide blocked aldosterone response to diazoxide but did not affect aldosterone response to exogenous ACTH to the same extent. Effect of diazoxide in insulin-treated rats was approximately similar to that of normal rats. Comparison of blood glucose concentration determined in normal, insulin treated and diabetic rats after different drug administration showed that there is no correlation between blood glucose level and the responses observed in serum hormone concentration. The results indicate that regulatory processes involved in the secretion of aldosterone are responsive to drugs affecting glibenclamide-sensitive K⁺ channels. *Iran. Biomed. J. 6 (2 & 3): 63-67, 2002*

Keywords: ATP-dependent K⁺ channel, Aldosterone, Blood glucose, ACTH

INTRODUCTION

The control of the synthesis and release of aldosterone is complex. Angiotensin II (AII) and potassium are the primary regulators of aldosterone secretion by adrenal zona glomerulosa cells [1]. ACTH produces a moderate stimulation of aldosterone release. Low plasma sodium or high plasma potassium concentration directly affects the zona glomerulosa cells of the adrenal, stimulating aldosterone release [2].

In addition to its independent regulatory role, K⁺ synergies with other adrenal secretagogues notably angiotensin II, [3]. Angiotensin II itself affect glomerulosa cell permeability to K⁺, causing a transient increase followed by a sustained decreases [4]. Aldosterone also involved in the regulatory process of potassium homeostasis [5]. Effects of extracellular K⁺ on aldosteronogenesis and effects of AN II on K⁺ fluxes must be exerted through K⁺ channels in the cell membrane [6]. Previous studies have shown that ionophores for monovalent cations

are potent inhibitors of aldosteronogenesis and A II [7]. On the other hand, there are many reports for involvement of ATP-sensitive potassium channels in pancreatic, cardiac, and vascular smooth muscle cells [8]. Cromakalim, a K⁺ channel activator, was also found to produce rennin secretion in healthy volunteers and to promote rennin secretion when applied directly to primary cultures of rat juxtaglomerular cells [9]. ATP-sensitive potassium channels also may involve in a rapid secretory mechanism of renal kallikrein by high potassium [10].

This observation provides a basis to study the effect of some ATP-dependent K⁺ channel affecting drugs on serum concentration of aldosterone. Since K⁺ channel affecting drugs used in our studies are known to change blood glucose level. Therefore, the effect of potassium channel openers was also determined in the presence of exogenous insulin and the effect of K⁺ channel blockers in streptozotocin-induced diabetic rats.

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MATERIALS AND METHODS

Male Wistar rats (150-200 g) ($n = 108$) were obtained from animal house of Pharmacology Department of Isfahan University of Medical Sciences. They had 12:12 h dark/light cycle. Glibenclamide, 20 mg/kg (Chimidar, Iran) and chlorpropamide 100 mg/kg (Razak, Iran) were dissolved in one ml of 0.05 M NaOH. Minoxidil, 10 mg/kg (Upjohn, USA) was dissolved in 0.1% of acetic acid and diluted with 0.9% NaCl and then administered intraperitoneally (i.p.). Streptozotocin, 75 mg/kg (Novo, Denmark), insulin, 0.5 I.U/kg (Novo, Denmark) and ACTH, 0.25 mg/kg (Organon, Holland) were diluted with saline and injected intraperitoneally.

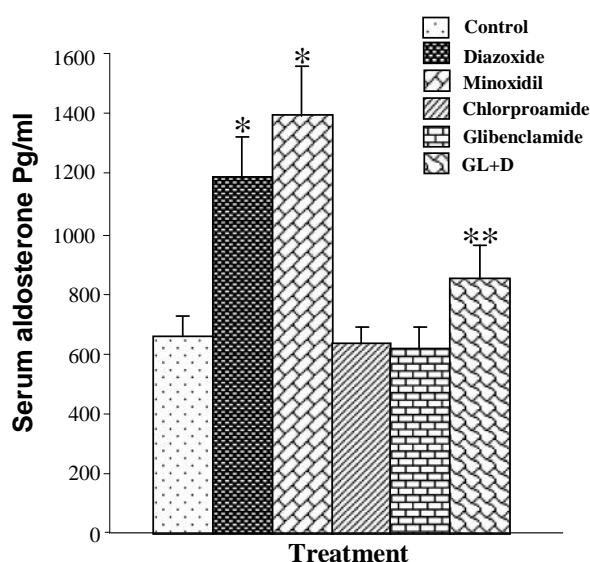


Fig. 1. Effect of K⁺ channel openers and blockers on serum aldosterone level in normal rats. Each bar indicates mean ± S.E.M of six rats per group. * $P < 0.05$ V.S non-treated rats; ** $P < 0.05$ V.S diazoxide treated rats.

Blood samples were obtained 60 minutes after the last injection. Rats receiving glibenclamide plus diazoxide, glibenclamide was administered 30 minutes before diazoxide. Sampling was performed by decapitation or by orbital sinus puncture from the eye. To avoid any diurnal changes in the biochemical parameters, sampling and experiments were performed at 8-9 a.m. each day. Samples were left to clot at room temperature for 15 minutes and then centrifuged at $628 \times g$ for 10 min. Serum layers were then frozen until used. Commercial Radioimmunoassay kits (Sorin, Italy) was used for determination of aldosterone [11]. In this method,

200 μ l of standard, control or serum samples were added to separate radiolabeled test tubes. After vortex-mixing tubes were incubated for 18-22 h at room temperature and after careful aspiration of incubation mixture, the radioactivity of tubes was measured by Gamma counter (Kontron, USA). Blood glucose concentration was measured by the method of Dubowski [12]. Briefly, 0.1 ml of fresh blood was added to 1.9 ml of 5% trichloroacetic acid (Merck, Germany), mixed and then centrifuged. O-toluidine reagent (2.5 ml) was added to 0.5 ml of the supernatant and left in a boiling water bath for 10 minutes. Tubes were removed and after cooling, the absorbance was measured by spectrophotometer (Perkin-Elmer, Germany) at 630 nm. Results are expressed as the mean \pm SEM. One way analysis of variance (ANOVA) and student's t -test was used for comparison of the means [13].

RESULTS

Effect of potassium channel openers and blockers on serum aldosterone levels in normal rats. Changes in mean serum aldosterone concentration after the administration of K⁺ channel openers and blockers are shown in Figure 1. The basal level of serum aldosterone was 659.32 ± 71.48 pg/ml. Diazoxide or minoxidil administration increased serum aldosterone concentration to 1188.53 ± 99.45 pg/ml and 1392.69 ± 177.83 pg/ml, respectively. After the injection of glibenclamide in diazoxide treated animals, serum aldosterone level was decreased from 1188.53 ± 99.45 pg/ml to 850.0 ± 75.08 pg/ml. Chlorpropamide or glibenclamide did not changed serum concentration of aldosterone in normal rats.

Effect of potassium channel openers and blockers on serum aldosterone level in the presence of exogenous ACTH. The effects of K⁺ channel affecting drugs on serum aldosterone level were studied in the presence of exogenous ACTH, which is a moderate stimulant of aldosterone releases. The aldosterone responses to ACTH and the effect of glibenclamide and diazoxide in the presence of exogenous ACTH are shown in Figure 2. Serum aldosterone concentration increased significantly ($p < 0.01$) one hour after ACTH administration (0.25 mg/kg). Pretreatment of rats with diazoxide produced about 15% increase in response to ACTH, but this change was not

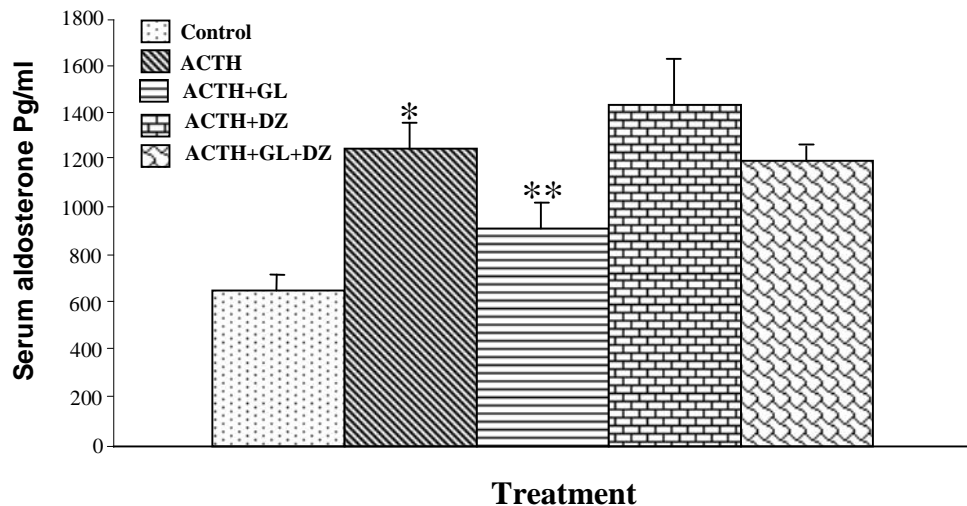


Fig. 2. Effect of diazoxide or glibenclamide on serum aldosterone response to exogenous ACTH. Each bar indicates mean \pm S.E.M of six rats per group * $P < 0.01$ V.S control rats; ** $P < 0.05$ V.S ACTH + diazoxide treated rats.

statistically significant. Mean serum aldosterone level was low after glibenclamide–ACTH administration, however it was not significant lower than ACTH administration alone. An aldosterone response to ACTH in the presence of glibenclamide was about 50% less than diazoxide pretreated animals ($p < 0.05$).

Effect of potassium channel openers on serum aldosterone level in insulin treated rats. We studied the hyperglycemia effect of diazoxide and minoxidil in the presence of exogenous insulin. As shown in Table-1, diazoxide and minoxidil in the presence of insulin caused a significant increase in aldosterone level ($P < 0.001$). Pretreatment with glibenclamide reversed this response to the diazoxide in insulin treated rats as compared to the control.

Effect of potassium channel blockers on serum aldosterone level in diabetic rats. To investigate the possible involvement of hypoglycemic effect of glibenclamide and chlorpropamide in hormonal responses of rats, we studied the effect of these drugs in streptozotocin-induced diabetic rats.

Four days after streptozotocin administration, the serum aldosterone level was increased 3 folds (Table 1). However, serum aldosterone concentration decreased significantly by chlorpropamide and glibenclamide K^+ channel blockers ($P < 0.01$ and $P < 0.05$, respectively). Pretreatment with diazoxide partially but not completely blocked the aldosterone response associated with glibenclamide administration in diabetic rats. Blood glucose concentrations due to the administration of some potassium channel affecting drugs in different conditions of our study were shown in Table 2.

Table 1. Serum aldosterone response to K^+ channel openers in insulin treated and to K^+ channel blockers in diabetic rats.

Treatment	Insulin Treated	Diabetics
Control	534.27 \pm 30.57	1561.23 \pm 125.07
Diazoxide (DZ)	1148.20 \pm 43.09***	
Minoxidil	1235.60 \pm 96.18***	
Glibenclamide (GL)		775.59 \pm 198.68*
Chlorpropamide		503.99 \pm 62.69**
GL+DZ	576.48 \pm 146.70	944.27 \pm 169.50

Diabetic rats received streptozotocin (75 mg/kg, I.P.) four days before experiments. Insulin treated rats received regular insulin (0.5 I.U/kg) one hour before drug treatment. Each value represents Mean \pm SEM of serum Aldosterone (pg/ml) levels of six rats. * $P < 0.05$ V.S related control; ** $P < 0.01$ V.S related control; *** $P < 0.001$ V.S related control

Table 2. Effect of K⁺ channel openers and blockers on blood glucose level in normal, insulin treated and diabetic rats.

Treatment	Blood glucose concentration (mg/dl)		
	Normal	Insulin Treated	Diabetics
Control	91.52 ± 3.62	85.02 ± 5.43	381.80 ± 43.50*
Diazoxide (DZ)	168.04 ± 10.87	119.36 ± 10.10	
Minoxidil	147.21 ± 8.69	109.22 ± 8.45	
Glibenclamide (GL)	44.27 ± 6.98		271.70 ± 31.17*
Chlorpropamide	58.19 ± 5.70		364.08 ± 60.50*
GL+DZ	126.98 ± 9.78	101.93 ± 8.69	470.64 ± 51.47*

**p*<0.001 vs related normal rats

DISCUSSION

In this study, potassium channel activators of diazoxide and minoxidil cause a significant increase in serum aldosterone level. Because of subcellular mechanism of potassium channel activation, direct effect of K⁺ channel openers on aldosterone secretion must be inhibitory. The renin secretory activity in contrast to most physiological secretory processes (e.g. insulin release) is increased when intracellular Ca²⁺ concentration decreased [14, 15]. Thus, if K⁺ channel activators can produce membrane hyperpolarization of juxtaglomerular cells, this may lead to a fall in cytosolic Ca²⁺ and, in turn, to an increase in renin secretion. Interestingly, studies with isolated rat kidney indicate that diazoxide stimulates renin secretion by a direct action [16]. The stimulation of renin secretion also is reported by potassium channel activator of cromakalim [9].

The minoxidil and diazoxide cause a smaller rise in serum aldosterone than expected from the fall in blood pressure [17, 18]. This sluggish aldosterone response may reflect direct inhibition of adrenal glomerulosa function, although *in vitro* studies have shown that minoxidil sulfate is not very potent inhibitor to aldosterone release. In this regard, Hadjokas and Goodfriend [6] also reported minoxidil sulfate, an active metabolite of minoxidil, was much less active in aldosterone secretion than vasoconstriction. In this regard, Von Nguyen *et al.* [19] reported that in the presence of potassium channel activator of cromakalim during AII infusion, aldosterone release was not affected, but arterial response to AII was blunted by cromakalim.

In this study, diazoxide administration in ACTH treated rats produces a mild additive effect in stimulating aldosterone secretion. This also potentiates the suggestion indicating diazoxide is not a potent inhibitor of aldosterone release.

Glibenclamide treatment in normal rats slightly decreases serum aldosterone concentration and moderately prevents the ACTH induced aldosterone elevation. This indicates glibenclamide sensitive K⁺ channels may be involved at least in some parts of the effect of ACTH-induced elevation in serum aldosterone level. This observation is in agreement with the previous report indicating glibenclamide (20 mg/kg I.V.) decreased basal plasma renin activity and strongly inhibited renin release evoked by diazoxide [21].

Previous study also showed that the basal level and angiotensin II stimulated aldosterone release inhibited by several K⁺ channel blockers including, glibenclamide, capsaicin, quinine and lidocaine [6]. In our study, inhibitory effect of glibenclamide and chlorpropamide on aldosterone release was more prominent in diabetic rats. Serum aldosterone concentration determined four days after streptozotocin administration was found to be significantly higher than that of normal rats. This is in agreement with the report of Kikkawa *et al.* [22] who showed higher levels of plasma renin activity, AII and plasma aldosterone concentration determined seven days after STZ administration.

This study demonstrated that under *in vivo* conditions glibenclamide had an antagonistic effect against the hyperaldosteronemic action of diazoxide. Antagonistic effect of diazoxide on renin secretion was also susceptible to blockade by glibenclamide [16, 20]. Furthermore, vasodilator effect of diazoxide mediated by ATP-sensitive potassium channels was susceptible to blockage by glibenclamide pretreatment [21, 23]. Eventually, at least some parts of decrease in serum aldosterone level by glibenclamide pretreatment could be accounted for prevention of diazoxide induced aldosterone release by glibenclamide. Results obtained for aldosterone after diazoxide administration in insulin treated rats seemed similar

to those of normal rats. This indicates ACTH or aldosterone response to diazoxide was not secondary to changes, which was observed in serum insulin level.

Based on the above observations, we suggest that aldosterone response to potassium channel affecting drugs not secondary to the change in serum glucose level. Also, ATP-sensitive potassium channels are involved in the regulatory process of aldosterone secretion.

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