EFFECTS OF GLIBENCLAMIDE, METFORMIN AND INSULIN ON THE INCIDENCE AND LATENCY OF DEATH BY OUBAIN-INDUCED ARRHYTHMIAS IN MICE

AZIZ YAZAR, GÜRBÜZ POLAT, ISMAIL UN, ADNAN LEVENT, AYŞE KAYGUSUZ, HANDAN ÇAMDEVİREN and KANSU BÜYÜKAFŞAR

Departments of aInternal Medicine, bBiochemistry, cPharmacology and dBiostatistics, Medical Faculty, Mersin University, Turkey

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This study was performed to investigate possible effects of glibenclamide, insulin and metformin on the death latency and incidence caused by a cardiac glycoside, ouabain. Mice of both sexes were injected with ouabain (i.p. 20 mg kg\(^{-1}\)), glibenclamide (s.c. 0.1–10 mg kg\(^{-1}\)), insulin (s.c. 0.3–3 U kg\(^{-1}\)) and metformin (i.p. 200 mg kg\(^{-1}\)) and combinations of the last three drugs with ouabain. Death latency was measured and lethality incidence was calculated. Death was assessed by visual observation. Plasma glucose level was evaluated from the tail blood. Glibenclamide (0.1 mg kg\(^{-1}\)) prolonged the latency from 11.3 ± 1.2 to 15.8 ± 1.8 min but failed to decrease the incidence of death. At higher doses (1–10 mg kg\(^{-1}\)) it had no effects on the latency or the incidence. 0.3 U kg\(^{-1}\) insulin decreased the incidence from 73.7 to 33.3% (\(p < 0.05\)) without affecting the latency. However the higher dose (3 U kg\(^{-1}\)) did not have any effects on the incidence or the latency. Oubain increased blood glucose level from 114.1 ± 3.8 (control) to 152.1 ± 5.3 mg dl\(^{-1}\). Metformin (200 mg kg\(^{-1}\)) did not affect either the latency or the incidence of death. While metformin did not decrease plasma glucose, insulin and higher doses of glibenclamide (1–10 mg kg\(^{-1}\)) markedly lowered glucose in blood. However, at the dose of 0.1 mg kg\(^{-1}\) glibenclamide did not alter the glucose level in the blood but prevented ouabain from increasing it. Insulin (0.3 U kg\(^{-1}\)) and, to some extent, glibenclamide (0.1 mg kg\(^{-1}\)) but not metformin could be effective antiarrhythmic agents against ouabain-induced arrhythmias.

KEY WORDS: arrhythmia, glibenclamide, insulin, metformin, ouabain.

INTRODUCTION

The Na\(^+\)/K\(^+\) ATP pump is a ubiquitous plasma membrane protein complex which couples the hydrolysis of one molecule of ATP to the exchange of three Na\(^+\) for two K\(^+\) ions, thus maintaining the normal gradient of these cations in animal cells [1], and this pump is selectively inhibited by cardiac glycosides [2]. Intoxication with these agents is invariably associated with cardiac arrhythmia. The arrhythmia is characterized by a prolonged PR interval and ventricular rhythm disorders [3]. On the other hand there is a functional interaction between Na\(^+\)/K\(^+\) ATPase and K\(_{\text{ATP}}\) channels as intracellular ATP level normally inhibits these channels [4]. In the heart the K\(_{\text{ATP}}\) channel appears to be activated during the ischaemic–hypoxic condition, and may be responsible for the increase of K\(^+\) efflux and shortening of the action potential duration favouring the genesis of ventricular fibrillation [5]. Inhibition of K\(_{\text{ATP}}\) channels by sulphonylureas attenuated the incidence of arrhythmias in isolated ischaemic/reperfused hearts from diabetic rats [6], decreased arrhythmogenesis during acute myocardial infarction in rats and reduced strophanthin cardiotoxicity in rabbits [7]. However, glibenclamide has been reported to exacerbate caesium-induced arrhythmias in anaesthetized dogs [8]. On the other hand insulin has been reported to stimulate ionic transport by the sodium pump and induce hyperpolarization in skeletal and cardiac muscle among other cells [9]. Overall, the aim of the present study is to examine possible effects of glibenclamide, metformin and insulin on the incidence and latency of mortality with the cardiac origin by ouabain in conscious mice.
MATERIALS AND METHODS

In the experiments 149 albino mice of both sexes weighing 20–30 g were used. Mice were divided into 14 different groups. One group served as control \((n = 20)\) with no treatment, where only plasma glucose level was detected, and the other groups received ouabain \((i.p. 20 \text{ mg kg}^{-1}, n = 19)\), glibenclamide \((s.c. 0.1–10 \text{ mg kg}^{-1}, n = 8–9)\), glibenclamide \((s.c. 0.1–10 \text{ mg kg}^{-1})\) plus ouabain \((i.p. 20 \text{ mg kg}^{-1}, n = 8–11)\), insulin \((s.c. 0.3–3 \text{ U kg}^{-1}, n = 8–10)\), insulin \((s.c. 0.3–3 \text{ U kg}^{-1})\) plus ouabain \((i.p. 20 \text{ mg kg}^{-1}, n = 9)\), metformin \((i.p. 200 \text{ mg kg}^{-1}, n = 10)\) and metformin \((i.p. 200 \text{ mg kg}^{-1})\) plus ouabain \((i.p. 20 \text{ mg kg}^{-1}, n = 10)\).

Following the drug injection \((\text{time zero})\) the mice were immediately placed in a transparent pot in order to make observations. Thereafter, death latency was measured, and percentage incidence was calculated. Death was assessed by visual observation. On the other hand blood glucose level was detected just after the death or 60 min cut-off time later in the survivors from the tail blood by use of glucose strips (Boehringer Mannheim, Germany).

All animals employed throughout the experiment were fasted for 12 h before the experimentation to standardize blood glucose variations. Room temperature was kept constant at 22 °C. During the experimentation any noise that could make the conscious animal subjects irritated was avoided.

Drugs

Glibenclamide, ouabain and dimethylsulphoxide (DMSO) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Metformin and neutral insulin (Actrapid®) were generous gifts from Ilsan Iltas Pharmaceutical Company (Turkey) and Novo Nordisk A/S (Bagsvaerd, Denmark) respectively. All drugs except for glibenclamide were dissolved or diluted in saline. Glibenclamide was dissolved in DMSO and diluted to the desired concentrations with saline.

Statistical analysis

Results are expressed as the mean ± SEM or median of \(n\) separate experiments. All statistical calculations were performed using a statistical software program (SPSS 9.05, CA, USA). Statistical comparisons were made by \(z\)-test, Mann–Whitney \(U\)-test or one-way analysis of variance followed by the Bonferroni post hoc test. A \(P\) value less than 0.05 was considered as significant.

RESULTS

Effect of glibenclamide, metformin and insulin on blood glucose level

Insulin at both doses \((0.3–3 \text{ U kg}^{-1})\) and glibenclamide \((1–10 \text{ mg kg}^{-1})\) dropped serum glucose level sharply, yet metformin \((200 \text{ mg kg}^{-1})\) had no effects. Lower dose of glibenclamide \((0.1 \text{ mg kg}^{-1})\) failed to decrease blood sugar level. Data are summarized in Table I.

Effect of ouabain on the death latency and incidence, and blood glucose level

20 mg kg\(^{-1}\) ouabain caused the death of 14 animals out of 19 with the percentage of 73.7 (Fig. 1). The death latency was 11.3 ± 1.2 minutes (Fig. 2). The blood glucose levels in the dead and survivors were 152 ± 5.3 and 142 ± 7.8 respectively which are different from the control value \((P < 0.05)\) (Table I).
Effect of glibenclamide on the ouabain-induced death latency and incidence

Glibenclamide at the intermediate and the higher doses (1 and 10 mg kg\(^{-1}\)) decreased neither the latency of death nor the incidence by ouabain (Figs 1 and 2). However, at the lower dose (0.1 mg kg\(^{-1}\)) it decreased death incidence from 73.7 to 60%, although this was not found significant (Fig. 1), but increased the latency from 11.3 ± 1.2 to 15.8 ± 1.8 min \((P < 0.05)\) (Fig. 2). At this dose of glibenclamide, blood glucose level was not different from the control but was from the ouabain group \((P = 0.05,\) Table I).

Effect of metformin on the ouabain-induced death latency and incidence

Metformin had no improving effect on ouabain-induced death latency or incidence (Figs 1 and 2). Neither did it alter blood glucose level (Table I).

Effect of insulin on the ouabain-induced death latency and incidence

Insulin at 3 U kg\(^{-1}\) increased the survival from 26.3 to 44.4% which is not significantly different (Fig. 1). However, the lower dose of insulin (0.3 U kg\(^{-1}\)) markedly increased the survival rate from 26.3 to 66.7% \((P < 0.05)\) (Fig. 1). At this dose, the life span of the dead mice was prolonged but found not to be significantly different from the control (Fig. 2). In these groups blood glucose levels were decreased (Table I).

DISCUSSION

We have examined whether insulin, a sulphonylurea, glibenclamide, and a biguanide derivative, metformin, have protective effects on a cardiac glycoside, ouabain-
induced death incidence and latency in mice. It has been well established that the cause of ouabain-induced death is severe arrhythmia in mice confirmed by electrocardiographic (ECG) data [10–12]. For that reason we only evaluated the duration and incidence of death by the glycoside in conscious mice rather than ECG data from anaesthetized mice. Opening of K\textsubscript{ATP} channels results in cardioprotective, as well as proarrhythmic effects as suggested earlier [13]. Glibenclamide prolonged ouabain-induced, arrhythmia-originated death duration (latency), but this effect was more predominant at lower dose rather than at higher dose, probably due to some untoward effects of glibenclamide. This sulphonylurea derivative is well known to act by blocking ATP-sensitive potassium channels. In several organ systems including the cardiovascular system, sulphonylurea receptors and functional K\textsubscript{ATP} channels have been identified. In the heart their role is not clear; an endogenous cardioprotective effect has been suggested [14]. Glibenclamide reduces both early K\textsuperscript{+} loss as well as ischaemic arrhythmias in the isolated perfused rat heart [15]. The activation of the ATP-sensitive potassium channel during myocardial ischaemia leads to potassium efflux, reductions in action potential duration and the generation of ventricular fibrillation [16]. A novel cardioselective inhibitor of the K\textsubscript{ATP}-sensitive potassium channel (HMR 1883), or glibenclamide prevented ventricular fibrillation in dogs. In contrast to glibenclamide, HMR 1883 did not elicit increases in plasma insulin and reduction in blood glucose [17]. In the present study the lowest doses of glibenclamide prolonged the latency of ouabain-induced lethality without changing plasma glucose level. However, it prevented ouabain from increasing the level of glucose in blood, suggesting that therapeutic action of glibenclamide might not be totally independent from the decreased blood glucose. This sulphonylurea has been known to increase plasma insulin level and reduction in blood glucose [17]. In this study the lowest dose of glibenclamide failed to decrease the plasma glucose level below the control value but lowered death duration by ouabain. Tolbutamide and carbutamide but not glibenclamide enhanced the incidence of digitalis intoxication in patients [18]. On the other hand, in rabbits glibenclamide decreased but tolbutamide and carbutamide increased strophanthidin toxicity [18]. In rats and guinea-pigs glibenclamide and tolbutamide were effective on arrhythmias induced by ischaemia and ouabain [19], supporting our findings. Although they obviously have different mechanisms of action, pretreatment with pinacidil and pretreatment with glibenclamide resulted in a very similar outcome concerning arrhythmias and sudden cardiac death (i.e. improvement of the survival rate) [20]. Glibenclamide has also an antiarrhythmic effect on ventricular fibrillation in non-insulin-dependent diabetics with acute myocardial infarction [21]. Furthermore, the proarrhythmic effect of cromakalim is abolished by glibenclamide, suggesting that the increased tendency to develop reperfusion arrhythmias is associated with the cromakalim-induced K\textsuperscript{+} efflux [22]. On the other hand, glibenclamide treatment aggravates ischaemia, and under the influence of glibenclamide, ischaemic preconditioning is no longer effective in reducing infarct size in the isolated perfused rabbit heart [23]. Glibenclamide also decreased coronary blood flow, which secondarily induced myocardial ischaemia and dysfunction [24]. Moreover, it did not significantly affect survival in chronically infarcted anaesthetized dogs [25]. In this study we also evaluated any possible effects of metformin, a second-line oral antihyperglycaemic agent. It did not diminish plasma glucose level. Neither did it prevent ouabain-induced mortality incidence or death duration.

On the other hand, insulin has been reported to prevent ventricular fibrillation in anaesthetized dogs [26]. Insulin stimulates ionic transport by the sodium pump and induces hyperpolarization which in most cases can be inhibited by cardiac glycosides or metabolic inhibition in skeletal and cardiac muscle among other cells [27]. Interestingly enough, administration of a physiological amount of insulin in mice (200 U g\textsuperscript{−1}) resulted in a ninefold increase of basal nitric oxide (NO) level [28]. NO has been demonstrated to act as a cardioprotective mediator in arrhythmias [29–31]. Whether possible NO release by insulin has any beneficial actions in protection against arrhythmia evoked by ouabain is not known in this study. One of the mechanisms of the cardioprotective effects of insulin may be due to the fact that insulin stimulates potassium transport into the cell which can counteract to the decreased influx of K\textsuperscript{+} by the inhibition of Na\textsuperscript{+}/K\textsuperscript{+} ATPase with ouabain. This effect is parallel with the decrease of plasma glucose level as insulin lowered the glucose level at both doses (0.3–3 U kg\textsuperscript{−1}). Accordingly, insulin can facilitate both the influx of K\textsuperscript{+} and glucose to target cells including myocytes. On the other hand, at higher insulin dose, it failed to prevent ouabain-induced lethality although it dramatically decreased the level of glucose. This indicates that insulin might act against arrhythmia via a way independent of decreased glucose level or, at higher dose, it has some deleterious effects on cardiac rhythm and thus enhances ouabain intoxication.

In conclusion, not metformin but insulin and, to some extent, glibenclamide could be effective antiarrhythmic agents in preventing ouabain-induced lethality.

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