行政院國家科學委員會補助專題研究計畫成果報告

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※　第 IA 及 III 類抗心律不整藥物對兩種延遲　※
※　整流鉀通道的作用：對反頻率依賴性的探討(2/2)　※
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計畫主持人：賴凌平

執行單位：國立臺灣大學醫學院

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一、中文摘要

人類極小鉀離子通道(minK)基因存在有多型性的現象，目前已知有兩種基因型，其所產生的蛋白相差一個胺基酸，其中 minK-G38 的第 38 個胺基酸為 glycine 而 minK-S38 的第 38 個胺基酸為 serine，這兩種極小鉀離子通道在電生理學上及藥物理學上的性質目前尚未明瞭，而這兩個基因型在國人的分佈機率也未曾被研究過，因而本研究的目的就在探索上述問題。

我們以聚合酶連鎖反應及體外轉錄的方式製造極小鉀離子通道的訊息核醣核酸，然後將它注射入蛙卵細胞中以產生鉀離子通道，並以雙電極電位鉗定的方式來研究並比較其電生理及藥理性質。我們並以聚合酶連鎖反應及限制酶斷片分析的方式來研究這兩個基因型在國人的分佈機率。我們的研究結果發現，這兩個極小鉀離子通道在電生理及藥理性質上並沒有顯著差異，它們都不會被 quinidine、 procainamide 和 sotalol 抑制，但會被 amiodarone 抑制，而 amiodarone 臨床上不具有反頻率依賴性，可能與抑制 minK 有關。我們也發現 minK-G38 的基因型在國人佔 69%而 minK-S38 的基因型佔 31%。

因此我們結論，目前的研究並未發現這兩種鉀離子在功能上的差異，而這個多型性可以在遺傳學及法醫學上被用作為基因標記。

關鍵詞：極小鉀離子通道，電位鉗定，分子生物學

Abstract

There are two kinds of human minK proteins differing from each other by one amino acid at the 38th position (glycine for minK-G38 and serine for minK-S38). However, the functional significance of the minK polymorphism is not clear and neither is the allele incidence.

The electrophysiological and pharmacological properties of both minK proteins were studied by heterologous expression of minK proteins in Xenopus oocytes. The allele incidence was determined by polymerase chain reaction and restriction fragment analysis. Human minK cDNA was obtained by polymerase chain reaction using genomic DNA from leukocytes as the template. MinK mRNA was obtained by in vitro transcription reaction. The mRNA was injected into Xenopus oocytes for heterologous expression. The two-electrode voltage clamp technique was performed to investigate the current induced by minK protein. We found that the two minK proteins did not differ significantly with regard to their electrophysiological and pharmacological properties. Both minK proteins were inhibited by amiodarone and were not inhibited by quinidine, procainamide and sotalol. The inhibitory effect of amiodarone on minK may be related to its clinical lack of reverse use-dependent effects. The incidences of both minK proteins were determined in 250 human subjects. We found that the allele incidences were 69% and 31% for minK-G38 and minK-S38 respectively.

The two kinds of minK proteins had similar electrophysiological and pharmacological properties. The allele incidences were 69% and 31% for minK-G38 and minK-S38 respectively. This polymorphism can be used as a marker in genetic studies.

Keywords: minK, potassium channel, molecular biology, voltage clamp study

二、緣由與目的

The minK protein was first cloned from rat kidney by Takumi and coworkers in 1987.[1] When expressed in Xenopus oocytes, minK induced a voltage-gated potassium current with very slow activation kinetics. The human counterpart was later on cloned by homology screening.[2] This protein is widely distributed in various animal species including mouse, guinea pig, and dog. The protein is expressed in numerous mammalian tissues such as kidney, duodenum, T-lymphocytes, uterus, inner ear and the heart.[2] The exact functional role of the minK protein has been the focus of many recent researches. It is now known that minK protein is the co-factor subunit of the slow component of the cardiac delayed rectifier potassium current (I_{Ks}).[3,4] It plays an important role in cardiac repolarization. A mutation of the minK gene has been reported to result in clinical congenital long QT syndrome.[5-7]

More recently, a genetic polymorphism was found in the human minK protein. Lai et al. reported that there are two kinds of human minK proteins different from each other by one amino acid.[8] MinK-S38 was the one cloned in 1989 by Murai et al. and has serine as the 38th amino acid. On the other

hand, minK-G38 was reported by Lai et al. and has glycine as the 38th amino acid. The functional differences between the two remained unknown. Since the 38th amino acid is at the extracellular part and serine has a hydroxy functional group while glycine does not, it is possible that the potassium current induced by these two proteins may be different in electrophysiological and pharmacological properties. Furthermore, the incidences of the two minK proteins in general population have not been extensively studied before. Therefore, we performed the present study to compare the electrophysiological and pharmacological properties of the two minK proteins by heterologous expression in the Xenopus oocytes. We also used the polymerase chain reaction and restriction enzyme digestion method to investigate the incidences of the two minK proteins in a hospital-based Chinese population.

三、結果與討論

Expression of minK protein in Xenopus oocytes

Outward current was recorded during a depolarizing voltage command in oocytes injected with minK mRNA, while no current was recorded in oocytes injected with distilled water (Figure 2). Therefore, the current recorded resulted from the mRNA injected. The shape of the induced current was characteristic of cardiac I_{Ks} current. We also found that the currents induced by minK-G38 and minK-S38 were similar.

Electrophysiological properties of the two human minK proteins

We measured the current-voltage (I-V) relationship, activation time constant, and deactivation time constant of the induced current. Figure 3 shows the relationship between voltage and the relative magnitude of current at the end of a five-second voltage command. We found that the two minK proteins did not differ significantly with regard to the I-V relationship. The channel was hardly activated until a voltage more positive than –20 mV. There was no inactivation for both minK-G38 and minK-S38. The activation and deactivation time constants are list in Table 1. There were no significant differences between the activation and deactivation time constants of the two minK proteins.

Pharmacological properties of the two human minK proteins

We tested the sensitivity of the two human minK proteins to quinidine, procainamide, sotalol, and amiodarone. Quinidine, procainamide and sotalol at 300μM had no inhibitory effects on the current induced by minK-G38 or minK-S38. On the other hand, amiodarone inhibited the current in a dose-dependent manner. The degree of inhibition was not significantly different between the two human minK proteins (Figure 4). With regard to the activation and deactivation time constants, all the four drugs had no effect on the time constants of the two minK proteins (Table 2).

Incidence of the two minK proteins in study population

The incidences of the two genetypes were determined in 250 patients. The incidence of minK-G38 homozygotes was 50.8% (127/250), minK-G38 homozygotes was 12.8% (32/250) and minK-S38/minK-G38 heterozygotes was 36.4% (91/250). The percentage of minK-G38 allele was 69% and that of the minK-S38 allele was 31%.

In the present study, we demonstrated that the currents induced by the two kinds of minK proteins do not differ significantly with regard to their electrophysiological and pharmacological properties. We also found that the allele incidences for minK-S38 and minK-G38 were 31% and 69% respectively. This polymorphism can reserve as a marker for genetic studies.

Functional significance of minK protein

MinK protein is a relatively small protein with only 130 amino acids and is therefore called minK (minimal K channel) protein.[1,2] Initial functional expression in Xenopus oocytes revealed that this protein induced a slowly activating, non-inactivating and slowly deactivating potassium current, very similar to I_{Ks}.[9] I_{Ks} is the slow component of the cardiac delayed rectifier potassium current. It has been reported that I_{Ks} can be detected in human ventricular and atrial myocytes at current[10,11] and mRNA levels.[3,4] Therefore, it contributes to cardiac repolarization in both atrial and ventricular levels and serves as a target for anti-arrhythmic drug therapy. However, the amino acid sequence of minK protein predicts that there is only one transmembranous segment. This feature is unique among all known ion channels and it has been doubtful that this protein is really an ion channel. Later on, it has been shown that minK is the co-factor subunit of I_{Ks}. It binds with another protein, KVLQT1,[12] to form the I_{Ks} channel.

Potential significance of minK polymorphism

There are a lot of polymorphisms in human genome. Some of the polymorphic sites have functional significance while others do not. For human minK protein, we demonstrated that the two kinds of minK proteins do not differ significantly with regard to their electrophysiological and pharmacological properties. Therefore, we cannot find any functional significance of the polymorphism at the present time. However, this polymorphism can be used as a marker in genetic studies and in forensic medicine. The minK gene has been mapped to chromosome 21 at 21q22.1-21q22.2.[13] There are a lot of known human genes located at human chromosome 21 such as amyloid precurser protein (APP),[14] carbonyl reductase (CBR3),[15] interleukin-10 receptor,[16] interferon type 1 and type 2 receptor,[16] human intersectin gene (ITSN).[17] There are also many diseases related to human chromosome 21 such as Down syndrome (Trisomy 21),[18] congenital long QT syndrome,[5-7] Alzhiemer's disease,[19] leukemia,[20,21] bipolar affective disorder[22] and so on. The minK gene polymorphism

can be utilized as a tool in studies related to the above genes and disorders. The polymorphism can be easily detected by polymerase chain reaction and restriction enzyme digestion. The allele incidences of 31% and 69% are also suitable for linkage analysis.

There are several limitations in this study. First, the I_{Ks} was obtained by heterologous expression of human minK protein in Xenopus oocytes. It is now known that KvLQT1 and minK protein together form the I_{Ks} channel.[3,4] The I_{Ks} channel in the present study was formed by human minK protein and endogenous KvLQT1 in the Xenopus oocytes. There might be interspecies differences in the properties of KvLQT1. However, the homology between human and Xenopus KvLQT1 is high (89-92%) and the induced current is very similar to I_{Ks} in human cardiac tissue with regard to its gating and kinetic properties.[4] Second, the study population in the present study is a hospital-based population. The patients included were those who suffered from cardiac disease and needed cardiac catheterization. The incidences of the alleles may be different from those of general population because linkage disequilibrium might exist between the gene and cardiac diseases or risk factors for cardiac diseases.

四、計畫成果自評

This is the first report regarding the functional study of minK gene polymorphism. In the present study, we demonstrated that the currents induced by the two kinds of minK proteins do not differ significantly with regard to their electrophysiological and pharmacological properties. Both minK proteins were inhibited by amiodarone and were not inhibited by quinidine, procainamide and sotalol. The inhibitory effect of amiodarone on minK may be related to its clinical lack of reverse use-dependent effects. This finding may guide future development of potassium channel blockers toward I_{Ks} inhibitors.

We also found that the allele incidences for minK-S38 and minK-G38 were 31% and 69% respectively. This polymorphism can reserve as a marker for genetic studies.

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