P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(1):96-99
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Electrophysiological effects of anthopleurin-Q on rat hepatocytes

Hong-Yi Zhou, Fang Wang, Ke-Qiang Zhang, Lan Cheng, Ji Zhou, Li-Ying Fu, Wei-Xing Yao

Hong-Yi Zhou, Fang Wang, Lan Cheng, Ji Zhou, Li-Ying Fu, Wei-Xing Yao, Department of Pharmacology, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Ke-Qiang Zhang, Department of Infectious Diseases, Jianghan Oil Field Central Hospital, Qianjiang 433124, Hubei Province, China
Correspondence to: Dr. Hong-Yi Zhou, Department of Pharmacology, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. zhouhy518@yahoo.com.cn
Telephone: +86-27-83692033
Received: 2003-05-11 **Accepted:** 2003-06-02

Abstract

AIM: To study the effects of AP-Q on CCl_4-induced acute liver injury, delayed outward potassium current (I_K), inward rectifier potassium current (I_{K1}) and calcium release-activated calcium current (I_{CRAC}) in isolated rat hepatocytes.

METHODS: A single dose of CCl_4 (10 μg/mL, ip) was injected to induce acute liver injury in rats. Serum aminotransferase activities were determined. Whole cell patch-clamp techniques were used to investigate the effects of AP-Q on delayed outward potassium current (I_K), inward rectifier potassium current (I_{K1}) and calcium release-activated calcium current (I_{CRAC}).

RESULTS: AP-Q (3.5 and 7 μg/kg) pretreatment significantly reduced ALT and AST activities. AP-Q 0.1-100 nM produced a concentration-dependent increase of I_K with EC_{50} value of 5.55±1.8 nM (n=6). AP-Q 30 nM shifted the I-V curve of I_K leftward and upward. CCl_4 4 mM decreased I_K current 28.6±6.5% at 140 mV. After exposure to CCl_4 for 5 min, AP-Q 30 nM attenuated the decrease of I_K induced by CCl_4 close to normal amplitude. AP-Q 0.01-100 nM had no significant effect on either inward or outward components of I_{K1} at any membrane potential examined. AP-Q 0.1-100 nM had no significant influence on the peak amplitude of I_{CRAC}, either, and did not affect the shape of its current voltage curve.

CONCLUSION: AP-Q has a protective effect on CCl_4-induced liver injury, probably through selectively increased I_K in hepatocytes.

Zhou HY, Wang F, Zhang KQ, Cheng L, Zhou J, Fu LY, Yao WX. Electrophysiological effects of anthopleurin-Q on rat hepatocytes. *World J Gastroenterol* 2004; 10(1): 96-99
http://www.wjgnet.com/1007-9327/10/96.asp

INTRODUCTION

Anthopleurin-Q (AP-Q) is a newly purified polypeptide toxin extracted from marine invertebrates, Anthopleura. Recently, it was reported that AP-Q had a cardiac antihypertrophy activity in rats[1]. It could attenuate the electrophysiological remodeling in rat hypertrophied myocardium. Carbon tetrachloride (CCl_4) was chosen as the hepatic toxicant to induce liver injury. After single CCl_4 administration, there was a progressive depolarization in rat liver cells between 3 and 27 h. At 48 h, a definite repolarization occurred. At 72 h, the surviving cells had resting potentials not significantly different from control values[2,3]. Hepatocytes isolated from CCl_4-induced cirrhotic rats had a lower membrane potential than normal healthy hepatocytes[4]. From the above, damaged hepatocytes have a depolarized membrane potential, but the mechanisms for maintenance of the resting membrane potential in hepatocytes are not well understood. Previous study showed that CCl_4 inhibited K^+ efflux through K^+ channel[5]. We investigated the effects of AP-Q on acute liver injury induced by CCl_4, and on membrane currents of isolated rat hepatocytes in order to explore the mechanisms against CCl_4-induced liver injury.

MATERIALS AND METHODS

Materials

AP-Q was provided by Qingdao Marine Biology Research Institute. It is a stable and basic polypeptide consisting of 40 amino acid residues, with a molecular weight of 4840 dalton, and a purity >99%. It is easily dissolved in distilled water. CCl_4 was from Beijing Chemical Plant.

Animals and treatments

Kunming strain mice of either sex weighing 18-22 g were used. The animals were divided into five groups, 12 animals each group. Acute liver injury was induced by intraperitoneal injection of a 0.1% (v/v) CCl_4 solution in olive oil at a dose of 10 mL per kg body weight. The control group was injected intraperitoneally with an equal volume of olive oil. AP-Q (3.5, 7 and 14 μg/kg) dissolved in saline was intraperitoneally injected once every day, for 7 days. CCl_4 treatment was given 1 h after the last dose. The same volume of saline was given to control group in the same manner. Animals of each group were killed by cervical dislocation 16 h after CCl_4 treatment, blood was collected from the orbital plexus and stored in a non-heparinized tube. Serum was separated from blood and stored at -20 ℃ until use.

Biochemical determinations

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using a commercial assay kit (Jian Cheng Co., Nanjing). Enzyme activities were expressed in Karman units (U/L).

Isolation of hepatocytes

Hepatocytes were isolated with the modified method reported by Seglen[6-8]. Briefly, adult Wistar rats of either sex (175±25 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The portal vein and inferior vena cava were cannulated and perfused with oxygenated Ca^{2+}-free Hank's solution 30 mL/min at 37 ℃ for 4-5 min followed by perfusion with Ca^{2+}-free Hank's solution containing collagenase (Type I, Sigma) (0.3 g/L) for 10 min. The liver was chopped in 10 mL Ca^{2+}-free Hank's solution. The cell suspension was filtered through a 200 mesh gauze and then centrifuged three times (50 g, 2 min) to separate liver cells. The cells were plated onto the coverslips and incubated in KB medium for 2 h and preserved in DMEM at 4 ℃.

Voltage-clamp recording

Whole-cell recordings were performed using a PC-II patch clamp amplifier (Huazhong University of Science and Technology). The recording chamber (1.5 mL) was perfused with the corresponding external solution. The pipettes were pulled in two stages from hard glass capillaries using a vertical microelectrode puller (Narishige, Japan). The electrode had a resistance of 2-5 MΩ for whole-cell recording when filled with electrode internal solution. All experiments were conducted at 22±2 ℃.

Solutions

Ca^{2+}-free Hank' s solution was prepared without Ca^{2+} and Mg^{2+}, containing (mM) NaCl 137, KCl 5.4, NaH_2PO_4 0.5, Na_2HPO_4 0.58, $NaHCO_3$ 4.16, and glucose 5.5 (pH 7.3). KB solution contained (mM) glutamic acid 70, taurine 15, KCl 130, KH_2PO_4 10, HEPES 10, glucose 11, egtazic acid 0.5, pH was adjusted to 7.4 with KOH. The external solution for recording I_{CRAC} contained (mM) NaCl 140, KCl 2.8, $CaCl_2$ 10, $MgCl_2$ 0.5, glucose 11, HEPES 10, pH was adjusted to 7.4 with NaOH. The internal solution for recording I_{CRAC} contained (mM) potassium-glutamate 145, NaCl 8, $MgCl_2$ 1, Mg-ATP 0.5, egtazic acid 10, HEPES 10, pH was adjusted to 7.2 with KOH. The external solution for recording I_K contained (mM) NaCl 144, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, glucose 5.5, HEPES 5, pH was adjusted to 7.4 with NaOH. The internal solution for recording I_K contained (mM) KCl 130, K_2ATP 5, creatine phosphate 5, HEPES 5, pH was adjusted to 7.2 with KOH. The same external and internal solutions for recording I_{K1} contained (mM) KCl 7, $MgCl_2$ 2, egtazic acid 1, potassium-glutamate 130, HEPES 10, pH was adjusted to 7.4 with KOH.

Statistics

The data were expressed as $\bar{x}\pm s$. Concentration-response relationship was calculated with Hill equation. Statistical significances were analyzed by Student' s *t*-test. *P* value <0.05 was considered significant.

RESULTS

Effect of AP-Q on serum ALT and AST activities in CCl_4-treated mice

Serum ALT and AST activities, indexes of liver cell damage of mice receiving a single CCl_4 injection were markedly increased 16 h after the injection. Pretreatment with AP-Q at various concentrations (3.5, 7 μg/kg) significantly reduced ALT and AST levels. By contrast, pretreatment with AP-Q (14 μg/kg) increased ALT and AST levels (Table 1), which were probably due to the intrinsic toxicity of AP-Q.

Table 1 Effects of pretreatment with AP-Q on serum ALT and AST levels in CCl_4-injected mice (*n*=12)

Drugs	ALT(U/L)	AST(U/L)
Olive oil	26.2±4.6	30.5±3.8
CCl_4	145.5±25.3[a]	110.1±20.6[a]
CCl_4+AP-Q 3.5 μg/kg	118.7±36.5[c]	90.8±29.2[c]
CCl_4+AP-Q 7 μg/kg	109.8±30.7[c]	88.9±30.7[c]
CCl_4+AP-Q 14 μg/kg	157.3±28.1	130.6±15.2

[a]*P*<0.05 *vs* control group, [c]*P*<0.05 *vs* CCl_4-treated group.

Effect of AP-Q on I_K

I_K was elicited by depolarizing pulse to +140 mV for 900 ms from a holding potential of -50 mV[9]. The current at the end of the test pulse was measured as the amplitude of I_K. At +140 mV, AP-Q 0.1-100 nM produced a concentration-dependent increase of the current, which was partially reversed after washout. EC_{50} value for AP-Q on I_K was 5.55±1.8 nM with the maximal increase of I_K up to 35.8±8.6% at AP-Q 30 nM (Figure 1B). The maximal increase in peak I_K did not further enhance when the concentration was raised to 100 nM.

Figure 1C shows the effects of AP-Q 30 nM on the steady-state I-V relationship for I_K generated by applying depolarizing steps from +30 mV to +140 mV for 900 ms with a 10 mV increment from a holding potential of -50 mV. AP-Q 30 nM shifted the I-V curve of I_K leftward and upward.

Cells were exposed to CCl_4 (4 mM) by addition of a concentrated aliquot (dissolved in DMSO) to the culture dish. The final concentration of DMSO was less than 0.1% (v/v). Under this condition, DMSO did not affect membrane current. Exposure to CCl_4 led to a significant decrease in I_K. The gradual decrease in I_K was detectable 1 min after CCl_4 exposure and the currents remained stable between 5-10 min. CCl_4 4 mM decreased I_K current 28.6±6.5% at 140 mV. After exposure to CCl_4 for 5 min, AP-Q 30 nM attenuated the decrease of I_K induced by CCl_4 close to normal amplitude (Figure 1. A, C).

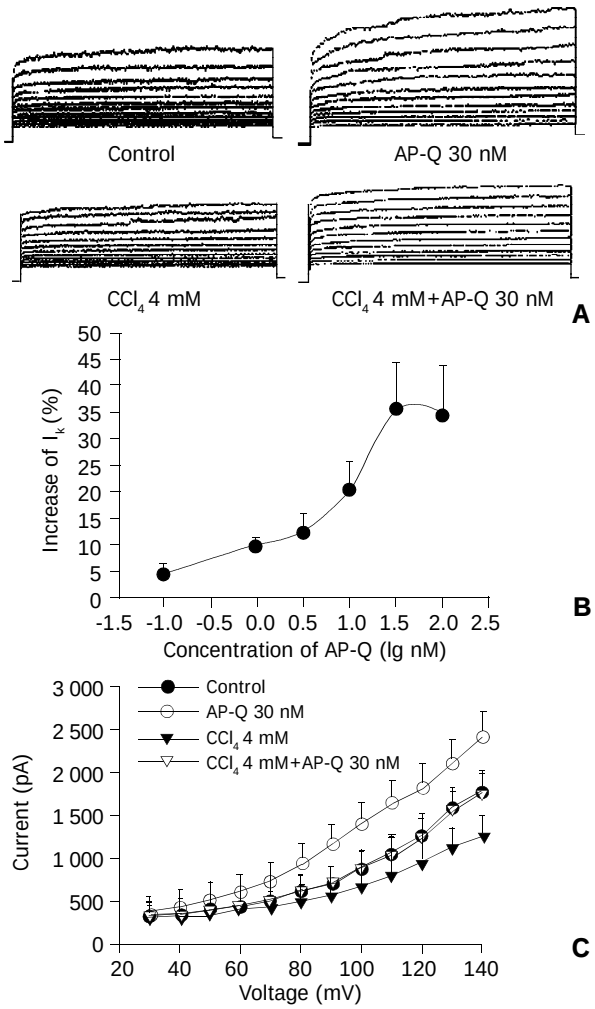

Figure 1 Effects of AP-Q on I_K. A: Family of I_K recorded with changes in the absence or presence of AP-Q 30 nM (upper) and family of I_K recorded with changes after CCl_4 administration in the absence or presence of AP-Q 30 nM (bottom). B: Concentration-response curve for the effects of AP-Q on I_K. *n*=6. C: I-V relationship of I_K under control (●), AP-Q 30 nM (○), CCl_4 4 mM (▼) and AP-Q 30 nM after CCl_4 4 mM administration (▽). The voltage steps used to elicit I_K are shown in the inset of panel (B). *n*=6.

Effect of AP-Q on I_{K1}

I_{K1} was elicited by a number of step pulses (40 ms) from the

holding potential of 0 mV to test potentials from -200 mV to +175 mV with a step of 10 mV[10]. The absolute value at the end of the test pulse was measured as the amplitude of I_{K1}. AP-Q 0.01-100 nM had no significant effect on either inward or outward components of I_{K1} at any membrane potential examined.

Effect of AP-Q on I_{CRAC}

I_{CRAC} was elicited for 200 ms from the holding potential of 0 mV to various potentials ranging from -100 mV to +80 mV with the step of 20 mV every 5 s[11]. The peak amplitude of I_{CRAC} was -495±127 pA (n=15) and the reversal potential of I_{CRAC} was about 0 mV, the current was steady and without run-down in 5 min. AP-Q 0.1-100 nM had no significant influence on the peak amplitude of I_{CRAC} and did not affect the shape of its current-voltage curve.

DISCUSSION

The results presented in this study demonstrated that pretreatment with AP-Q 3.5 μg/kg and 7 μg/kg had a protective effect on CCl_4-induced acute liver injury, reflected by changes in serum AST and ALT activities. However, pretreatment with AP-Q 14 μg/kg aggravated the toxicity of CCl_4, probably due to the intrinsic toxicity of AP-Q.

On the basis of its liver protective effect, we investigated the effects of AP-Q on membrane potassium and calcium currents of isolated rat hepatocytes to explore its mechanisms against CCl_4-induced liver injury.

Potassium channels are ubiquitous in eukaryotic cells and play roles in resting membrane potential, frequency of action potential, membrane potential repolarization rates and cell functions. It is noteworthy that small conductance Ca^{2+}-activated K^+ channel played a fundamental role in liver injury[12]. Progesterone induced cholestasis at least in part by inhibition of inwardly rectifying K^+ channel[13,14]. ATP-sensitive K^+ channel regulated proliferation of liver cells[15].

We found CCl_4 decreased I_K in a time dependent manner. The decrease of I_K might partly contribute to membrane depolarization. CCl_4-induced hepatocytes injury paralleled with membrane depolarization in damaged hepatocytes[2-4]. Similarly, nicotine blocked multiple types of K^+ currents, elevating the risk for cardiovascular disease and sudden coronary death associated with smoking[16]. Phenytoin (PHT) blocked I_K resulting in hypoxia-reoxygenation damage[17].

As cells became depolarized, hepatocellular substrates uptake decreased. Conversely, as cells became hyperpolarized, uptake increased[4,18,19]. Similar to vascular tissues where K^+ channels represent a protective and adaptive mechanism, opening of K^+ channels in liver cells could be beneficial at the early stages of injury since membrane hyperpolarization would stimulate electrogenic uptake of substrates important for regeneration of cellular ATP stores[20]. In addition, K^+ efflux was necessary for recovery from cell swelling[21,22]. Hyperpolarization of the hepatic parenchymal cell membrane played a role in hepatic cytoprotection[23]. Glycine has been reported to have several beneficial effects, including protection against hepatic toxicity induced by anoxia, oxidative stress, and various toxic agents at cell, organ, and systemic levels. Glycine activated glycine-sensitive chloride channels could lead to hyperpolarization of hepatic parenchymal cell membranes, block the increase in $[Ca^{2+}]_i$ due to agonists released during stress to protect against liver injury. Sea anemone toxins were common potassium channel modulators[24-26]. In our study, we investigated the effects of AP-Q on K^+ channels. AP-Q increased I_K in a concentration-dependent manner, resulting in hyperpolarization of hepatic parenchymal cell membranes, and AP-Q attenuated the decrease of I_K induced by CCl_4 close to normal amplitude of I_K, which might be beneficial for CCl_4 induced acute liver injury. AP-Q had no effect on I_{K1}. It is interesting to note that major sea anemone toxin possessed potassium channel blocking properties except *Bainh* increased I_{K1}[26].

Calcium has been demonstrated to play an important role in liver damage[27]. An early disturbance in hepatocellular Ca^{2+} homeostasis might be involved in hepatocellular damages induced by CCl_4[28-30]. Hepatocytes have been found to be short of voltage-dependent Ca^{2+} channels[31] but to possess I_{CRAC}[10, 32-34]. In our previous study, we found tetrandrine and palmatine could inhibit I_{CRAC}, protecting hepatocytes from calcium overload[35,36]. AP-Q had no influence on I_{CRAC}, suggesting that AP-Q did not affect the influx of extracellular Ca^{2+}.

In conclusion, AP-Q has a protective effect on CCl_4-induced liver injury, probably by selectively increased I_K, which in part counteracts the membrane depolarization in CCl_4-induced liver damages.

REFERENCES

1 **Zhou J**, Fu LY, Yao WX, Xia GJ, Jiang MX, Wang JR, Wu ZG, Qian LL. Effects of anthopleurin-Q on myocardial hypertrophy in rats and physiologic properties of isolated atria in guinea pigs. *Acta Pharmacol Sin* 2002; **23**: 924-929

2 **Wands JR**, Smuckler EA, Woodbury WJ. Transmembrane potential changes in liver cells following CCl_4 intoxication. *Am J Pathol* 1970; **58**: 499-508

3 **Olson JR**, Hosko MJ, Fujimoto JM. Alterations in the liver cell transmembrane potential following $CC1_4$ and bile salt treatment of rats. *Life Sci* 1979; **25**: 2043-2050

4 **Burczynski FJ**, Wang GQ, Minuk GY, Rosser B. Altered transmembrane ionic flux in hepatocytes isolated from cirrhotic rats. *J Hepatol* 1999; **30**: 492-497

5 **Thalhammer T**, Kaschnitz R, Mittermayer K, Haddad P, Graf J. Organic solvents increase membrane fluidity and affect bile flow and K^+ transport in rat liver. *Biochem Pharmacol* 1993; **46**: 1207-1215

6 **Seglen PO**. Preparation of isolated rat liver cells. *Methods Cell Biol* 1976; **13**: 29-83

7 **Zhang GL**, Wang YH, Teng HL, Lin ZB. Effects of aminoguanidine on nitric oxide production induced by inflammatory cytokines and endotoxin in cultured rat hepatocytes. *World J Gastroenterol* 2001; **7**: 331-334

8 **Wang YJ**, Li MD, Wang YM, Ding J, Nie QH. Simplified isolation and spheroidal aggregate culture of rat hepatocytes. *World J Gastroenterol* 1998; **4**: 74-78

9 **Li JM**, Cui GY, Liu DJ, Cui H, Chang TH, Wang YP, Zhang KY. Effects of N-methyl berbamine on delayed outward potassium current in isolated rat hepatocytes. *Zhongguo Yaoli Xuebao* 1998; **19**: 24-26

10 **Henderson RM**, Graf J, Boyer JL. Inward-rectifying potassium channels in rat hepatocytes. *Am J Physiol* 1989; **256**(6 Pt 1): G1028-G1035

11 **Cui GY**, Li JM, Cui H, Hao LY, Liu DJ, Zhang KY. Effects of calcium channel blockers on calcium release-activated calcium currents in rat hepatocytes. *Zhongguo Yaoli Xuebao* 1999; **20**: 415-418

12 **Barfod ET**, Moore AL, Lidofsky SD. Cloning and functional expression of a liver isoform of the small conductance Ca^{2+}-activated K^+ channel SK3. *Am J Physiol Cell Physiol* 2001; **280**: C836-C842

13 **Waldegger S**, Beisse F, Apfel H, Breit S, Kolb HA, Haussinger D, Lang F. Electrophysiological effects of progesterone on hepatocytes. *Biochim Biophys Acta* 1995; **1266**: 186-190

14 **Breit S**, Kolb H, Apfel H, Haberland C, Schmitt M, Haussinger D, Graf J, Lang F. Regulation of ion channels in rat hepatocytes. *Pflugers Arch* 1998; **435**: 203-210

15 **Malhi H**, Irani AN, Rajvanshi P, Suadicani SO, Spray DC, McDonald TV, Gupta S. K_{ATP} channels regulate mitogenically induced proliferation in primary rat hepatocytes and human liver cell lines. Implications for liver growth control and potential therapeutic targeting. *J Biol Chem* 2000; **275**: 26050-26057

16 **Garateix A**, Vega R, Salceda E, Cebada J, Aneiros A, Soto E. BgK anemone toxin inhibits outward K^+ currents in snail neurons. *Brain Res* 2000; **864**: 312-314

17 **Wang H**, Shi H, Wang Z. Nicotine depresses the functions of multiple cardiac potassium channels. *Life Sci* 1999; **65**: 143-149
18 **Azarbayjani F**, Danielsson BR. Phenytoin-induced cleft palate: evidence for embryonic cardiac bradyarrhythmia due to inhibition of delayed rectifier K^+ channels resulting in hypoxia-reoxygenation damage. *Teratology* 2001; **63**: 152-160
19 **Lidofsky SD**, Fitz JG, Weisiger RA, Scharschmidt BF. Hepatic taurocholate uptake is electrogenic and influenced by transmembrane potential difference. *Am J Physiol* 1993; **264**(3 Pt 1): G478-G485
20 **Weisiger RA**, Fitz JG, Scharschmidt BF. Hepatic oleate uptake. Electrochemical driving forces in intact rat liver. *J Clin Invest* 1989; **83**: 411-420
21 **Wondergem R**, Harder DR. Transmembrane potential and amino acid transport in rat hepatocytes in primary monolayer culture. *J Cell Physiol* 1980; **104**: 53-60
22 **Howard LD**, Wondergem R. Effects of anisosmotic medium on cell volume, transmembrane potential and intracellular K^+ activity in mouse hepatocytes. *J Membr Biol* 1987; **100**: 53-61
23 **Wang K**, Wondergem R. Mouse hepatocyte membrane potential and chloride activity during osmotic stress. *Am J Physiol* 1992; **263**(4 Pt 1): G566-G572
24 **Qu W**, Ikejima K, Zhong Z, Waalkes MP, Thurman RG. Glycine blocks the increase in intracellular free Ca^{2+} due to vasoactive mediators in hepatic parenchymal cells. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G1249-G1256
25 **Gilquin B**, Racape J, Wrisch A, Visan V, Lecoq A, Grissmer S, Menez A, Gasparini S. Structure of the BgK-Kv1.1 complex based on distance restraints identified by double mutant cycles. Molecular basis for convergent evolution of Kv1 channel blockers. *J Biol Chem* 2002; **277**: 37406-37413
26 **Lanigan MD**, Pennington MW, Lefievre Y, Rauer H, Norton RS. Designed peptide analogues of the potassium channel blocker ShK toxin. *Biochemistry* 2001; **40**: 15528-15537
27 **Salinas EM**, Cebada J, Valdes A, Garateix A, Aneiros A, Alvarez JL. Effects of a toxin from the mucus of the Caribbean sea anemone (Bunodosoma granulifera) on the ionic currents of single ventricular mammalian cardiomyocytes. *Toxicon* 1997; **35**: 1699-1709
28 **Lafuente NG**. Calcium channel blockers and hepatotoxicity. *Am J Gastroenterol* 2000; **95**: 2145
29 **Hemmings SJ**, Pulga VB, Tran ST, Uwiera RR. Differential inhibitory effects of carbon tetrachloride on the hepatic plasma membrane, mitochondrial and endoplasmic reticular calcium transport systems: implications to hepatotoxicity. *Cell Biochem Funct* 2002; **20**: 47-59
30 **Recknagel RO**. A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci* 1983; **33**: 401-408
31 **Huang ZS**, Wang ZW, Liu MP, Zhong SQ, Li QM, Rong XL. Protective effects of polydatin against CCl_4-induced injury to primarily cultured rat hepatocytes. *World J Gastroenterol* 1999; **5**: 41-44
32 **Sawanobori T**, Takanashi H, Hiraoka M, Iida Y, Kamisaka K, Maezawa H. Electrophysiological properties of isolated rat liver cells. *J Cell Physiol* 1989; **139**: 580-585
33 **Rychkov G**, Brereton HM, Harland ML, Barritt GJ. Plasma membrane Ca^{2+} release-activated Ca^{2+} channels with a high selectivity for Ca^{2+} identified by patch-clamp recording in rat liver cells. *Hepatology* 2001; **33**: 938-947
34 **Gregory RB**, Barritt GJ. Evidence that Ca^{2+}-release-activated Ca^{2+} channels in rat hepatocytes are required for the maintenance of hormone-induced Ca^{2+} oscillations. *Biochem J* 2003; **370** (Pt 2): 695-702
35 **Zhou HY**, Wang F, Cheng L, Fu LY, Zhou J, Yao WX. Effects of tetrandrine on calcium and potassium currents in isolated rat hepatocytes. *World J Gastroenterol* 2003; **9**: 134-136
36 **Wang F**, Zhou HY, Cheng L, Zhao G, Zhou J, Fu LY, Yao WX. Effects of palmatine on potassium and calcium currents in isolated rat hepatocytes. *World J Gastroenterol* 2003; **9**: 329-333

Edited by Zhu LH and Wang XL