Shi YL *et al* / Acta Pharmacol Sin 2003 Jan; 24 (1): 22-30

Ion-channels in human sperm membrane and contraceptive mechanisms of male antifertility compounds derived from Chinese traditional medicine[1]

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KEY WORDS spermatozoa; ion channels; lipid bilayers; spermatogenic cells; male antifertility agents; Chinese herbal medicine

ABSTRACT

Ion channel plays a key role in maturation, capacitation and acrosome reaction of sperm. However, as it is difficult to record channel currents from a mature mammal sperm directly by patch-clamp technique, there were no basic data on the types and properties of the channels in human sperm until the method reconstituting the channels into bilayer was used. By reconstituting the channel proteins isolated from sperm membrane into phospholipid-forming bilayer, we have characterized several kinds of Ca^{2+}-, Na^+-, K^+-, and Cl^--permeable channels with different conductance and properties in human sperm membrane. To study the channels in spermatogenic cells is another approach to understand the ion-channels in mature sperm. The cell is used as a model to analyze the effects of male antifertility agents on Ca^{2+}-channel. To date, several male contraceptives derived from Chinese traditional medicine have been attached worldwide interest, a lot of compounds have been purified from them, and the antifertility effects of some compounds were demonstrated. We studied the effects of gossypol and several compounds isolated from *Tripterygium wilfordii* on Ca^{2+} channel in mouse spermatogenic cells and found that each of them inhibited the channel and sperm acrosome reaction at a proximate concentration, suggesting that the inhibition of Ca^{2+} channels may be one mechanism of the antifertility effects of these contraceptives.

ESSENTIAL ROLE OF ION-CHANNELS IN SPERM MATURATION AND CAPACITATION AND SPERM-OOCYTE INTERACTION

Ionic fluxes through ion-channels are crucial in sperm maturation, capacitation, and the initiating process of gamete interaction. Modifying the activities of ion-channels is one important way of reproductive hormones to regulate the fertility process and of male antifertility agents to induce contraceptive effects. The role of Ca^{2+} channels in these processes was noticed firstly. It was reported that the existence of extracellular Ca^{2+} was essential in acrosome reaction (AR), a rise in Ca^{2+} concentration in sperm occurred during AR and an inhibition in the maturation, capacitation and AR of sperm was observed in Ca^{2+} channel blockage[1-3]. Regarding the actions of monovalent cations, it has been known that sperm maturation was modified directly by the changes in the concentration of Na^+ and K^+ in the

[1] Project supported by the National Natural Science Foundation of China (No 38388013, 38970831, 39480005, and 39870197).
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Received 2002-06-13 Accepted 2002-10-14

epididymis. The effluxes of K^+ and H^+ were observed during AR and the progesterone-evoked AR was inhibited in a Na^+-free medium[4]. Chloride ions have been demonstrated to be necessary for AR evoked by progesterone or zona pellucida (ZP). The progesterone-evoked AR was accompanied with Cl^- efflux, and the blockers of $GABA_A$ receptor/Cl^- channel, picrotoxin and bicuculline have been found to inhibit AR significantly[5-8]. These facts indicate a crucial participation of ion-channels in maturation, capacitation and sperm-oocyte interaction and the existence of various kinds of ion-channels in human sperm membrane. However, due to the small size, high differentiation, complex geometry, and motility of mammalia sperm, intracelluar recording or patch-clamp technique is very difficult to be used satisfactorily to record the channel current and characterize the types and properties of the ion-channel in intact human sperm. This is why there were no basic data on ion-channels in human sperm until ion channel-bilayer reconstitution method was introduced to the study field.

FUNCTIONAL RECONSTITUTION OF ION-CHANNEL PROTEIN IN BILAYER MEMBRANE

Ion-channels are the kind of membrane proteins and when incorporated into lipid bilayer membrane they provide the pathway for ion transmembrane transport. Thus, when ion-channel proteins or fragments of biomembrane are incorporated into artificial bilayer membrane that separated two water phases, the channel-mediated currents can be recorded and characterized by patch-clamp amplifier. That is the ion channel-bilayer reconstitution method being progressively developed and perfected in the recent years[9,10]. By the method, we have recorded and analyzed several kinds of single events mediated by the channels formed by pore-formers derived from the toxins of snakes and fish[11-13], antibiotics[14,15], lectin[16,17], type II ribosome-inactivating protein[18], and chloroplast ATP synthase CF_0-CF_1[19,20]. All the electrophysiological data involving the type and property of the channels in human sperm membrane are resulted from the studies using the channel-bilayer reconstitution method.

Procedures to form artificial phospholipid bilayer exploit the several conformation that phospholipid molecules can adopt in water. These conformations arise as a consequence of the amphiphilic nature of lipids, composed of a polar head group and a non-polar hydrocarbon tail. The two-compartment system with a septum to suppport the bilayer was used firstly. Various reconstitution techniques were introduced to incorporate channel proteins into bilayers. The recent implementation of the method makes use of glass patch-clamp pipettes to assemble the bilayer film at the tip of the electrode. A direct application of patch-clamp technique to reconstitution consists of the formation of giga-seals against the surface of giant liposome containing ion-channels[9] (Fig 1).

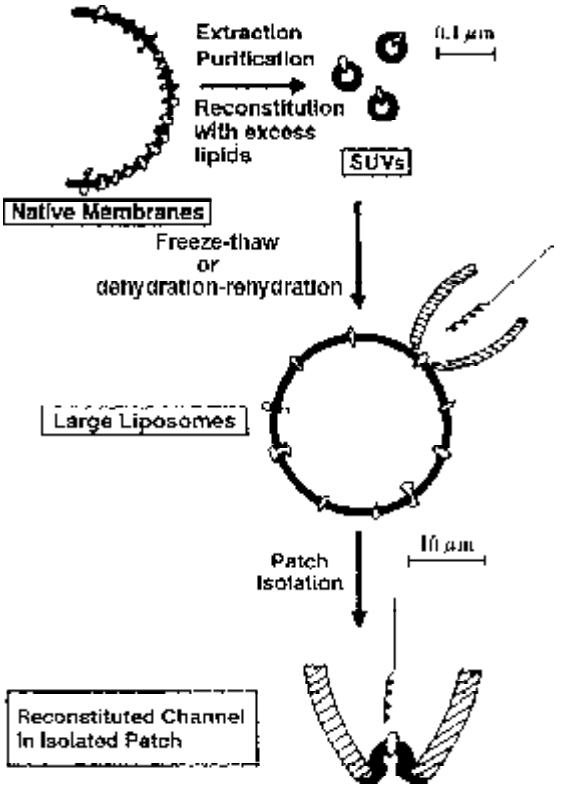

Fig 1. Strategy for isolated patch recording of ion-channels into giant liposomes. The giant liposomes were formed by dehydration-rehydration or freeze-thaw procedures[9].

TYPES AND PROPERTIES OF ION-CHANNELS IN HUMAN SPERM MEMBRANE

Ca^{2+} channels Using the two compartment system, the ion-channel proteins in human sperm were reconstituted into phospholipid artificial bilayer and single channel currents were recorded in a $CaCl_2$ solution system. We found that there existed two kinds of Ca^{2+}-permeable channels with different conductance at least. One with open-subconductance was voltage-dependent and its open probability (P_o) increased upon depolarization. Another was non-voltage dependent. The reversal potentials of them were close to the Nernst potential of Ca^{2+} electrode and both were completely in-

hibited by nifedipine and verapamil although with different efficacious concentration. These results[21] indicated that the recorded channel currents were carried by Ca^{2+} (Fig 2). Meanwhile, the infertile human sperm samples were also investigated and an abnormal activity in Ca^{2+} channels was observed, ie, the channels with an extremely long open time and no returning to their previous close state. The abnormal channel activity had never been detected in the samples derived from health donors, suggesting that there were some variations in the channel protein conformation of infertile sperm[22].

Monocation channels Tetraethylammonium (TEA)-sensitive K^+ channels and tetrodotoxin (TTX)-sensitive Na^+ channels were detected from human sperm membrane by the similar reconstituiton system[21-23]. For further analysis of the Na^+-channels, we changed reconstitution method to ressembling the proteins into liposomes and then fusing the liposomes into giant liposomes (diameter >10 μm) by dehydration-rehydration procedure[24]. By this method, two kinds of Na^+ channels with different conductance were observed. Both of them were TTX-sensitive and strongly rectifying and with a reversal potential near the Nernst potential of Na^+ electrode (Fig 3). In a symmetric solution system (200 mmol/L NaCl), their unit conductance were 60 pS and 140 pS, respectively. The large one exhibited subconductance state between close and full open and burst activity with flicker transition. By constructing the open or close dwell time distribution histogram and fitting it by exponential function, we obtained two time constants for each state, implying that there were two open and two close states in the large Na^+ channel at least. In addition, another Na^+-channels with a unit conductance somewhat between the mentioned two channel events was also observed. The I-V curve for the middle one was linear, with a conductance of 70 pS[24]. By the way, similar to abnormal Ca^{2+}-channel activity, no-close property of monovalent cation channels was observed in infertile sperm sample[22].

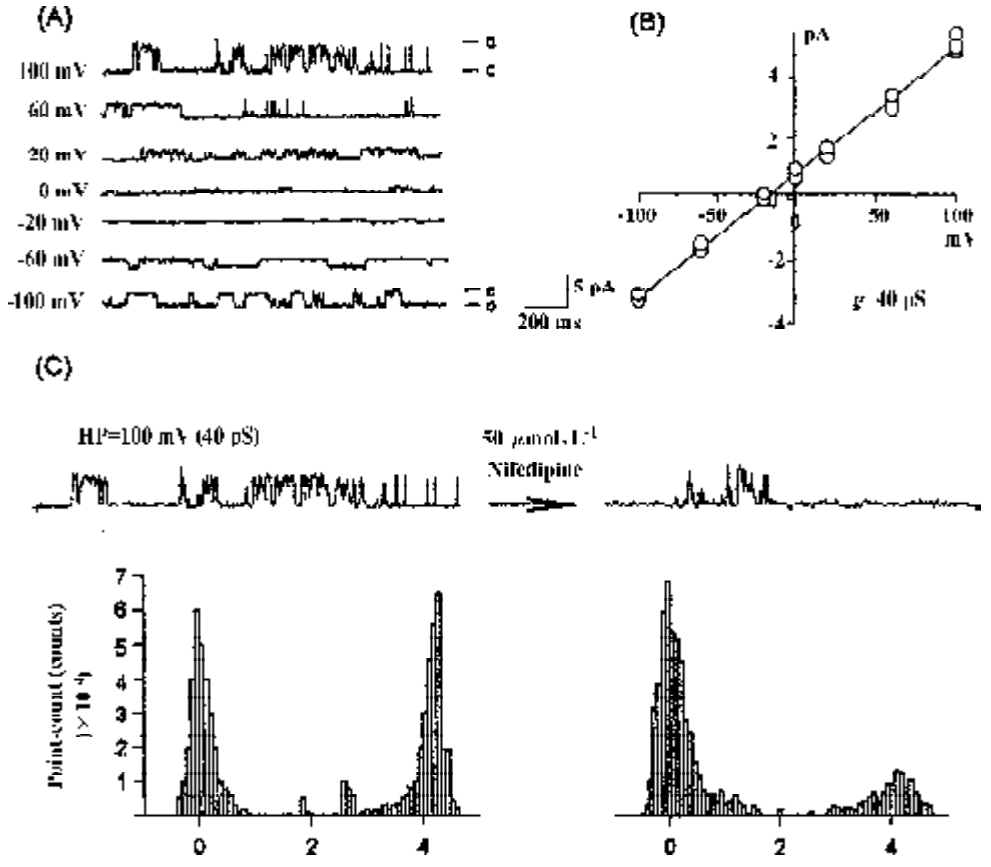

Fig 2. Single channel currents recorded in $CaCl_2$ solutions and their depression by nifedipine. A, Channel events observed at seven different holding voltages with "-c" indicating the closed state of the channel. B, Current-voltage relationship obtained from three same experiments. C, Depressive effects of Ca^{2+} channel blocker nifedipine (50 m mol/L) on Ca^{2+} channel current. The concentration gradient was 50 (*cis*) to 10 (*trans*) mmol/L $CaCl_2$[21].

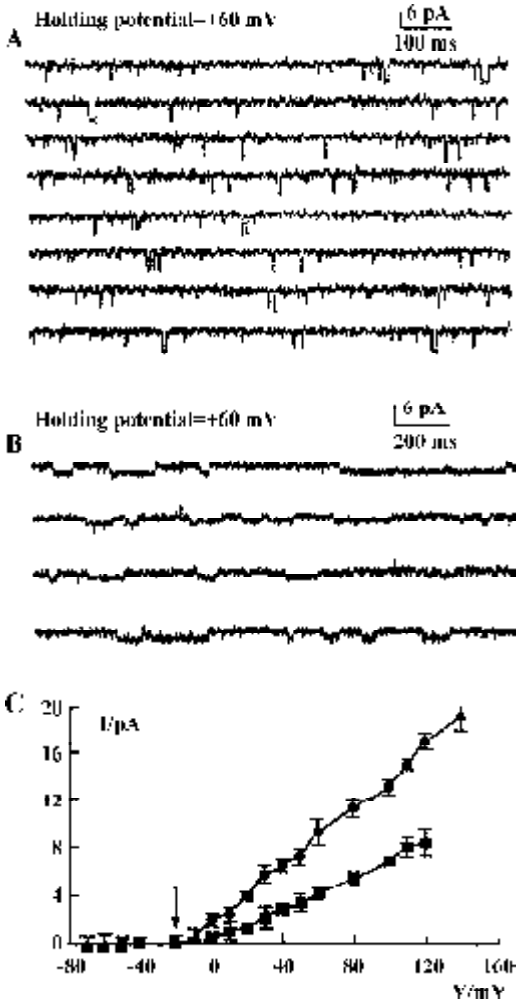

Fig 3. Single-channel currents recorded from giant liposome in which channel proteins derived from human sperm membranes were reconstituted. A and B are respectively from two excised patches, demonstrating two Na^+ channels with different conductance and open probability. C shows current-voltage relationship of the large (●, *n*=9) and the small (■, *n*=3) Na^+ channels with reversal potential ($^-$) of -18 mV[24].

Cl^- channels By the reconstitution method mentioned above, ie, reassembling ion channel into giant liposome, three kinds of Cl^- channels with unit conductance of 74.1 pS, 117.0 pS, and 144.7 pS, respectively were characterized in an *N*-methyl-*D*-glucamine (NMDG) solution sysytem[25]. All of them could be blocked by 4-acetamido-4'-isothiocyanatostilbene-2',2'-disulfonic acid (SITS), a Cl^--channel blocker, in a concentration-dependent manner. By constructing open or close dwell time histogram and then fitting it with exponential function, two time constants were obtained in both open and close states. The burst activity and subconductance states were often observed in the three Cl^- channels. Their open was voltage-dependent (Fig 4) as demonstrated by the following facts: 1) The channel P_o showed a maximum at -100 mV and decreased upon depolarization; 2) The channel open frequency was high at negative potential and obviously low at positive potential.

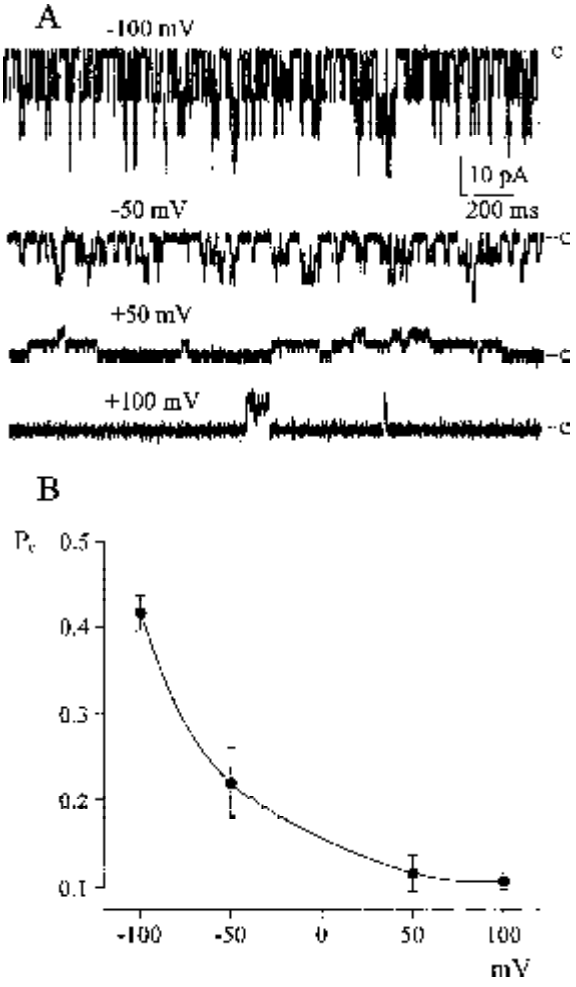

Fig 4. Voltage-dependence of human sperm Cl^- channel. A, Original recordings from a three-channel patch; B, P_o (open probability)-voltage relationship. The unit conductance of channel is 117 pS in bath 100// pipette 200 mmol/L NMDG-Cl solution. The results were obtained from a patch[25].

SPERMATOGENIC CELL, AN USEFUL MODEL IN ANALYZING CONTRACEPTIVES ACTION ON Ca^{2+}-CHANNELS

Spermatogenic cells are the developmental precursors of spermatozoa. Pachytene spermatocytes and round spermatids are two prominent cell types of spermatogenic cells obtained from diploid meiotic and haploid postmeiotic stages of spermatogenesis. These cells with diameter about 20 μm in mice are suitable for patch

clamp recording. Now, it is accepted that spermatozoa are terminally differentiated cells without the ability to synthesize protein, and the ion-channels expressed during spermatogenesis will retain in mature sperm. So, besides channel-bilayer reconstitution, to characterize the ion-channels in the spermatogenic cells is another method to approach the ion-channels in mature sperm.

Up to date, Ca^{2+}-, Cl^--, and K^+-permeable channels were observed by whole-cell patch clamp recording in mammal spermatogenic cells[4,26-28]. The K^+ channels were sensitive to TEA and their density progressively decreased during spermatogenesis[26]. The Ca^{2+}-channels with low voltage-activated, fast activating and inactivating, high Ni^{2+}-sensitivety showed the properties of T-type channels[26-28]. In view of the essentiality of Ca^{2+} in the function of sperm and important role of T-type Ca^{2+} channels in the regulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during capacitation and the acrosome reaction in sperm[4], the spermatogenic cell is often used as a model system of mature sperm to study the effects of male antifertility agents on Ca^{2+} channels and hence to explore their contraceptive mechanisms.

STUDIES ON MALE CONTRACEPTIVES DERIVED FROM CHINESE TRADITIONAL MEDICINES

There are a lot of male contraceptives in Chinese traditional medicines, such as seeds of *Gossypium herbaceum* L, roots of *Tripterygium wilfordii* Hook f, and whole-plant of Begonia yunnanensis Lévl or Sphagnum teres Aongstr. Up to now, many antifertility compounds were purified and identified from these Chinese traditional medicines, and the contraceptive effects of some of them, for example gossypol purified from cotton seeds, the extracts and compounds isolated from roots of *Tripterygium wilfordii* Hook f were demonstrated in the studies performed on human and animals. Meanwhile, their antifertility mechanisms were analyzed.

Gossypol Gossypol, a polyphenolic compound, when it was administered via mouth induced infertility in rat, hamster, guinea pig, monkey, and man. The effect could be reversible after withdrawal of the drug [29]. Although the idea that to use gossypol as a clinical long-term contraceptive drug was disputed due to its side-effects, such as hypokalemia, weakness, and irreversible azoospermia, recent studies showed that the drug could cause contraception and without side-effects at a low dosage when it was cooperated with steroid hormone contraceptives[30]. Previous data demonstrated that gosspol affected sperm capacitation, motility, AR, and ability to penetrate oocytes[31]; induced morphological changes in spermatogenic cells and spermatozoa, such as inducing the damage in sperm nucleus and acrosome, even head-tail separation after injecting drugs into epididymis[29]; reversibly decreasing the amount of tubulin and dynein in spermatocyte and spermatid, hence inhibiting the transformation from round- to elongated-head spermatid[32]; impairing mitochondrial sheath, probably by uncoupling oxidative phosphorylation or inhibiting lactate dehydrogenase. It was also reported that after treatment with gossypol, the animals caused hypophysectomy-like symptoms, ie, a block of hormone regulation on spermatogenesis[33].

Cyclic adenosine monophosphate (cAMP) plays a key role in sperm maturation and sperm-oocyte fusion. It was reported that gossypol inhibited adenylate cyclase and cAMP phosphodiesterase, two key enzymes were involved in the synthesis and hydrolysis of cAMP, and resulted in the reduction of intracellular cAMP, and then down-regulated the activities of cAMP-dependent protein kinases and the biosynthesis of androgens[29,34].

Acrosome reaction, an initial event necessary to sperm-oocyte fusion, was found to be Ca^{2+}-dependent. Based on that gossypol concentration-dependently inhibited sperm AR as well as Ca^{2+}-uptake and Ca^{2+}-ATPase in human spermatozoa membrane vesicles[31,35], we studied the effects of gossypol on Ca^{2+} channels in mouse spermatogenic cells by whole-cell recording. The results showed that at a similar concentration to inhibit AR (5-80 μmol/L), gossypol concentration-, time-dependently, and irreversibly inhibited T-type Ca^{2+} channels. The time to block the channels was progressively shortened as the gossypol concentration was increased from 5 to 80 μmol/L (Fig 5). Moreover, this drug did not affect the activation of the channels, while increased their inactivation time constant in concentration- and time-dependent manner. It was also observed that the inhibitory effect on the T-type Ca^{2+} current did not correlate with signalings mediated by G proteins and tyrosine phosphorylation, suggesting that the effect may result from a direct action on the channels. Gossypol had no obvious effects on Cl^- currents[36]. The facts that gossypol inhibited AR and Ca^{2+} channel at a similar concentration, indicated that the gossypol-induced blockade of Ca^{2+} channels could be responsible for its inhibition on AR and hence for the antifertility

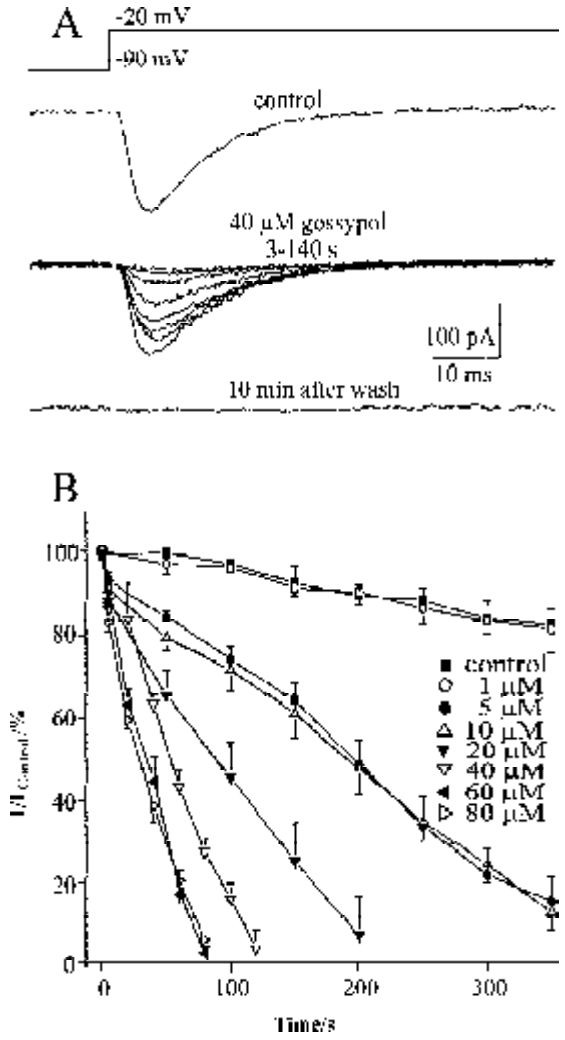

Fig 5. Gossypol-induced inhibition of T-type Ca^{2+} currents. A, an example showing the original records (every 20 s) in a spermatogenic cell before and after gossypol application and after washing. B, Time course of the current decrease after the application of different concentrations of gossypol. Each point represents the mean±SEM of 3-5 experiments similar to that shown in A[36].

activity of the drug. These results suggest a possibility to use gossypol locally as an emergency vaginal contraceptive drug.

Extract and compounds derived from *Tripterygium wilfordii* Hook f The plant containing alkaloids, diterpenoids, triterpenoids, and sesqueterpenes, was used in Chinese traditional medicine to cure rheumatoid arthritis, chronic nephritis, ankylosing spodylitis and various skin diseases. Its male antifertility effect was found as a side effect in treating the diseases[37]. It was observed that when oral administration with GTW (10 $mg \cdot kg^{-1} \cdot d^{-1}$), a water-chloroform extract from the plant, all male rats became infertile in 8 weeks while the histology of the seminiferous tubules and serum testosterone level were unaltered[37]; oral administration with 20-30 $mg \cdot kg^{-1} \cdot d^{-1}$ induced infertile in man without side effects. It has been shown that GTW acts mainly on spermatogenic cells, so its antifertility effect may result from an inhibition of sperm transformation and maturation via its selective action on spermatogenic cells in testis[38].

Up to now, many compounds including triptolide, tripchlorolide, tripdiolide, demethylzeylasteral, celastrol, and *L*-epicatechin, *etc*, have been isolated from GTW[37]. Some of them have been demonstrated to have an antifertility effect, in which tripchlorolide was the most effective. Previous studies showed that GTW could induced damages of microtubule, microfilament, and plasma membrane, even head-tail separation of sperm, and decrease the motility and the concentration of sperm in the cauda epididymis dramatically[37]. Following the treatment with these compounds, a reduction of total basic nuclear protein and an increase of total histone/protamine ratio were observed in sperm, indicating an inhibition of nuclear protein transition and a decrease of protamine levels in sperm[39].

It was reported that celastrol and demethylzeylasteral inhibited progesterone-induced AR concentration-dependently[40,41] (Tab 1). The blocking effects of GTW, demethylzeylasteral, celastrol, and *L*-epicatechin

Tab 1. A comparison of the efficacious dosages of some male antifertility compounds to inhibit both sperm AR and spermatogenic cell Ca^{2+} current.

	Demethylzeylasteral (μg/mL)	Celastrol (μg/mL)	*L*-epicatechin (μg/mL)	GTW (μg/mL)	Gossypol (μmol/L)	Amiloride (μmol/L)
Ca^{2+} current	≥1, IC_{50}=8.8[41]	≥5[41]	≥1000[42]	≥1, IC_{50}=6.4[42]	≥5[36]	IC_{50}=245[44]
AR	≥2.5[41]	≥0.5[40]			≥5[31]	≥100[41]
Oral contraception			2000 $mg \cdot kg^{-1} \cdot d^{-1}$[43]	10 $mg \cdot kg^{-1} \cdot d^{-1}$ [38]	12-40 $mg \cdot kg^{-1} \cdot d^{-1}$[29]	

on mouse spermatogenic cell Ca^{2+} channels were demonstrated in our recent studies[41,42] (Fig 6). Demethylzeylasteral and GTW inhibited the channels concentration-dependently and reversibly with a similar IC_{50}. Both of them enhanced the time constants of activation and inactivation of the channels. The effective concentration of *L*-epicatechin to inhibit the channels was 1000 times higher than that of GTW and demethylzeylasteral. *L*-epicatechin prolonged the inactivation of the channel at a higher concentration. The inhibition of Ca^{2+} channels by celastrol was similar to that by gossypol, showing time- and concentration-dependence. From that these compounds inhibited spermatogenic cell Ca^{2+} channels and Ca^{2+} influx-evoked sperm AR at a proximate concentration, it was suggested that the inhibition of Ca^{2+} channels might be one mechanism of antifertility effect of the compounds. Previous studies showed that the dosages of the immuno-suppressive effect of GTW and these compounds were 5-10 times higher than those of their antifertility effects[37]. The further studies to elevate safety factor and eliminate side-effect of these drugs are performing in some laboratories.

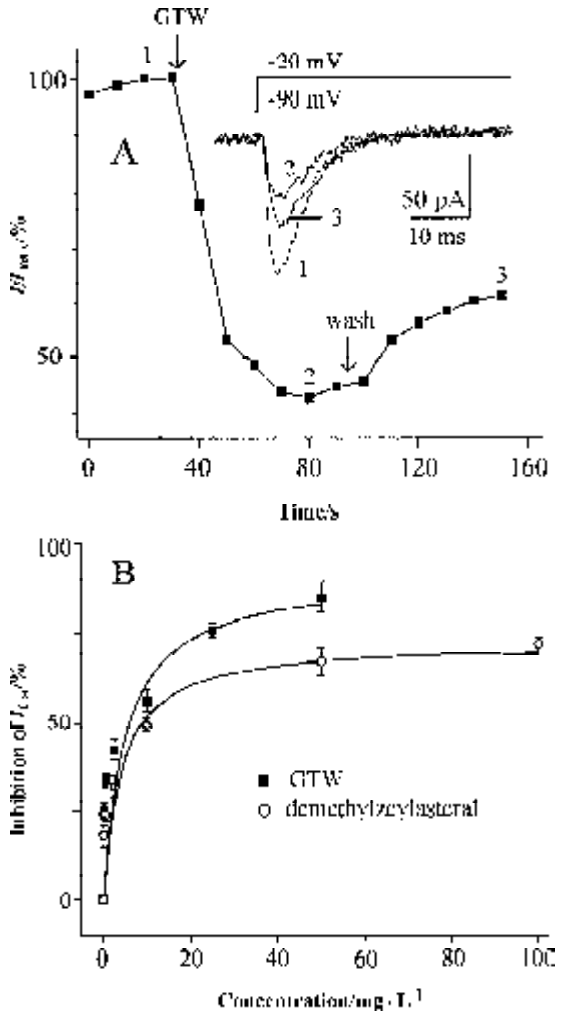

Fig 6. GTW and demethylzeylasteral-induced inhibition of T-type Ca^{2+} currents in mouse spermatogenic cells. A, Change of the Ca^{2+} current after 10 mg/mL GTW application and washing. Insert: whole-cell original recording. B, Concentration-response relationships. Peak currents were normalized with respect to the control. Each point represents the mean±S.E.M. of 5 experiments[42].

In conclusion, ion-channel plays a crucial role in sperm maturation, capacitation, and AR. Several kinds of Ca^{2+}-, Na^{+}-, K^{+}- and Cl^{-}-channels have been successfully identified by channel protein-lipid bilayer reconstitution method. Mouse spermatogenic cell, as a model of mature sperm, has been used to analyze the effects of male contraceptive drugs on Ca^{2+}-channels. The antifertility effects of gossypol and some compounds isolated from *Tripterygium wilfordii* Hook f have been demonstrated and the inhibition of T-type Ca^{2+} channels could be responsible for their antifertility activity.

REFERENCES

1 Anand RJK, Kanwar U, Sanyal SN. Calcium channel antagonist verapamil modulates human spermatozoal functions. Res Exp Med (Berl) 1994; 194: 165-78.
2 Kanwar U, Anand RJK, Sanyal SN. The effect of nifedipine, a calcium channel blocker, on human spermatozoal functions. Contraception 1993; 48: 453-70.
3 Ma XH, Shi YL. Ion channels of mammalian and human sperm membrane. Prog Physiol Sci 1998; 29: 109-14.
4 Darszon A, Labarca P, Nishigaki T, Espinosa F. Ion channels in sperm physiology. Physiol Rev 1999; 79: 481-510.
5 Bai JP, Shi YL. Two types of Cl^{-} channel in the plasma membranes of mammalian and human sperm and their involvement in acrosome reaction. Reprod Contraception 1999; 19: 203-06.
6 Wistrom CA, Meizel S. Evidence suggesting involvement of a unique human sperm steroid receptor/Cl^{-} channel complex in the progesterone-initiated acrosome reaction. Dev Biol 1993; 159: 679-90.
7 Sabeur K, Edwards DP, Meizel S. Human sperm plasma membrane progesterone receptor(s) and the acrosome reaction. Biol Reprod 1996; 54: 993-1001.
8 Melendrez CS, Meizel S. Studies of porcine and human sperm suggesting a role for a sperm glycine receptor/Cl^{-} channel in the zona pellucida-initiated acrosome reaction. Biol Reprod 1995; 53: 676-83.
9 Coronada R, Labarca P. Reconstitution of single ion-channel molecules. TINS 1984; 7: 155-60.
10 Shi YL. Reconstitution of ion channels of excitable membrane in lipid bilayers. Biol Commun 1989; 1: 5-8.
11 Shi YL, Edwards C, Lazarovici P. Ion selectivity of the channels formed by pardaxin, an ionophore, in bilayer

membranes. Natural Toxins 1995; 3: 151-5.
12 Shi YL, Wang WP, Zhuo XL. Ion-channels formed at lipid bilayer by the toxic component isolated from the roes of naked carp. Acta Biophys Sin 1995; 11: 357-61.
13 Shi YL, Wang WP, Zhang H, XuK. Channel-forming activity at planar lipid bilayer of the membrane active polypeptide B from venom of *Bungarus Fasciatus*. Acta Physiol Sin 1992; 44: 533-40.
14 Shi YL, Wang WP, Zhou YC. Ionic channels formed in lipid bilayer membrane by aureofuscin, a polyene antibiotics. Acta Physiol Sin 1991; 43: 128-33.
15 Cui JJ, Tang SB, Shi YL, Wang WP. Selective transport of Li^+ and Na^+ through lipid bilayers by nanhumycin. Acta Physiol Sin 1994; 46: 231-7.
16 Shi YL, Wang WP, Zhang H, Wang KY, Guo M. Cation channels formed in lipid bilayer by pinellia ternata lectin. Acta Physiol Sin 1992; 44: 142-8.
17 Zhang H, Shi YL. Cation selectivity of the channels formed at planar lipid bilayer by pinellia ternata lectin. Sci Sin (B) 1994; 37: 547-56.
18 Zhang GP, Shi YL, Wang WP, Liu WY. Cation channel formed at lipid bilayer by cinnamomin, a type II ribosome-inactivating protein. Toxicon 1999; 37: 1313-22.
19 Ma XH, Wei J, Shi YL. Study on ion-conductive property of the chloroplast H^+-ATPase reconstituted into planar lipid bilayer. Acta Biophys Sin 1995; 11: 497-502.
20 Ma XH, Shi YL. Effects of ADP, DTT and Mg^{2+} on the ion-conductive property of chloroplast H^+-ATPase (CF_0-CF_1) reconstituted into bilayer membrane. Biochem Biophys Res Commun 1997; 232: 461-3.
21 Shi YL, Ma XH. Ion channels reconstituted into lipid bilayer from human sperm membrane. Mol Reprod Dev 1998; 50: 354-60.
22 Ma XH, Shi YL. A patch clamp study on reconstituted calcium permeable channels of human sperm plasma membran. Acta Physiol Sin 1999; 51: 571-9.
23 Chan HC, Zhou TS, Fu WO, Wang WP, Shi YL, Wong PY. Cation and anion channels in rat and human spermatozoa. Biochim Biophys Acta 1997; 1323: 117-29.
24 Zhang GP, Bai JP, Shi YL. Na^+-permeable channels of human sperm membrane reassembled into giant liposome. Chin Sci Bull 2001; 46: 1085-9.
25 Bai JP, Shi YL. A patch-clamp study on human sperm Cl^- channel reassembled into giant liposome. Asian J Androl 2001; 3: 185-91.
26 Hagiwara S, Kawa K. Calcium and potassium currents in spermatogenic cells dissociated from rat seminiferous tubules. J Physiol 1984; 356: 135-49.
27 Arnoult C, Villaz M, Florman HM. Pharmacological properties of the T-type Ca^{2+} current of mouse spermatogenic cells. Mol Pharmacol 1998; 53: 1104-11.
28 Santi CM, Darszon A, Arturo H. A dihydropyridine-sensitive T-type Ca^{2+} current is the main Ca^{2+} current in mouse primary spermatocytes. Am J Physiol 1996; 271: C1583-93.
29 Shi QX, Tso WW, Friend DS. Gossypol inhibition of spermatogenesis, sperm motility and metabolism. In: Mohri H, editor. New horizons in sperm cell research. Tokyo: Japan Sci Soc Press/New York: Gordon and Breach Sci Publication; 1987. p 389-408.
30 Gu ZP, Mao BY, Wang YX, Zhang RA, Tan YZ, Chen ZX, *et al*. Low dose gossypol for male contraception. Asian J Androl 2000; 2: 283-7.
31 Shi QX, Friend DS. Gossypol-induced inhibition of guinea pig sperm capacitztion *in vitro*. Biol Reprod 1983; 29: 1027-32.
32 Teng CS. Reversible changes in cellular and microtubular tubulin in spermatogenic cells after gossypol treatment. Contraception 1997; 55: 183-8.
33 Oko R, Hrudka F. Comparison of the effects of gossypol, estradiol-17 beta and testosterone compensation on male rat reproductive organs. Biol Reprod 1984; 30: 1198-207.
34 Vishwanath R, White IG. Inhibition of ram sperm adenylate cyclase by gossypol and interaction with manganese. In: Mohri H, editor. New horizons in sperm cell research. Tokyo: Japan Sci Soc Press/New York: Gordon and Breach Sci Publication; 1987. p 431-7.
35 Kanwar U, Batla A, Sanyal S, Minocha R, Majumdar S, Ranga A. Gossypol inhibition of Ca^{2+} uptake and Ca^{2+}-ATPase in human ejaculated spermatozoal plasma membrane vesicles. Contraception 1989; 39: 431-45.
36 Bai JP, Shi YL. Inhibition of T-type Ca^{2+} currents in mouse spermatogenic cells by gossypol, an antifertility compound. Eur J Pharmacol 2002; 440: 1-6.
37 Qian SZ, Xu Y, Zhang JW. Recent progress in research on *Tripterygium*: a male antifertility plant. Contraception 1995; 51: 121-9.
38 Zheng JR, Fang JL, Xu LF, Gao JW, Guo HZ, Li ZR, *et al*. Effect of total glycosides of *T. wilfordii* on reproductive organs of male rats. Acta Acad Med Sin 1985; 7: 256-9.
39 Dai WP, Liu P, Han YH, Chen XM, Fei RR, Xue SP. The effect of *Tripterygium wilfordii* monomers T_4, T_7, T_{15} and Triptolide on rat nuclear protein. Acta Acad Med Sin 1994; 16: 20-3.
40 Yuan YY, Gu ZP, Shi QX, Qin GW, Xu RS, Cao L. *In vitro* inhibition of celastrol on spermatozoa fertilization ability of guinea pig. Acta Pharm Sin 1995; 30: 331-5.
41 Bai JP, Shi YL, Shi QX, Fang X. Effects of demethylzeylasteral and celastrol on Ca^{2+} channels in mouse spermatogenic cells and their possible relevance to sperm acrosome reaction. CAPS New Commu 2002; 21: (in press).
42 Bai JP, Shi YL. Inhibition of Ca^{2+} channels in mouse spermatogenic cells by male antifertility compounds from *Tripterygium wilfordii* Hook. f.. Contraception 2002; 65: 441-5.
43 Chen ZZ, Hu YB, Fang NN, Cai YC, Ling QS. Antifertility effect of *L*-epicatechin on male rats. Chin Pharm Bull 1988; 23: 46-7.
44 Arnoult C, Kazam IG, Visconti PE, Kopf GS, Villaz M, Florman HM. Control of the low voltage-activated calcium channel of mouse sperm by egg ZP3 and by membrane hyperpolarization during capacitation. Proc Natl Acad Sci USA 1999; 96: 6757-62.