



# Influence of Glutamate Receptor Antagonist Mk801 on Human Sperm Movement and Impregnating Ability



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## Abstract

N-methyl-D-aspartate (NMDA) receptor subunit 2B (NR2B) was found to be expressed in human sperm in our previous study. Here, we extended this observation to evaluate the potential pharmacological actions of NR2b-targeting reagents on sperm by using a sperm-activity assay and a sperm-cervical mucus-contact test. It was shown that MK-801 [(+)-5-methyl-10, 11-dihydroxy-5H-dibenzo(a, d) cyclohepten-5, 10-imine], a well-known potent NR2B antagonist, markedly interfered with the direction and speed of sperm movement as well as apparently inhibited the ability of sperm to pass through uterus mucilage. Thus, MK-801 acts by inhibitions of sperm motility and fertilization, which are possibly via NR2B antagonism. The present findings indicate that NR2B-targeting compounds such as MK-801 have the potential to be used clinically for decreasing the probability of human pregnancy and would meet the contraceptive need of people.

**Keywords:** Glutamate receptor antagonist; MK-801; Sperm; Impregnating ability

## Introduction

Glutamate receptors are classified into ionotropic and metabotropic receptors as defined primarily by their pharmacological and molecular profiles. The ion-type receptors are subdivided into NMDA, kainate (KA), and  $\alpha$ -Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors [1,2], each of which is capable of coupling ion channels directly and thereby known to possess a ligand-gating property. Until now, it has been proved that the family of sodium ion-dependent glutamate receptors is composed of GLAST, GLT1, EAAC1, EAAT4, and EAAT5 subtypes [3-7]. Moreover, these ionotropic glutamate receptors (ligand-gated ion channels) can be further divided into various subtypes such as NR1/NR2A/NR2B/NR2C/NR2D (the five subtypes for NMDA receptors), GluR1 /GluR2/ GluR3/GluR4 (for AMPA receptors), and GluR5/GluR6/GluR7/ KA1 /KA2 (for KA receptors) [1,4], according to their homology sequence and ligand affinity. With regard to the NMDA receptors, the two major subunits NR1 and NR2 are expressed at high abundance in tissues; wherein, NR2 family (NR2A, NR2B, NR2C,

and NR2D subunit) has recently received much attention in the field of pharmacology. These NR2 subunits are expressed alternatively at different developmental stages, wherein the expression of NR2B has been proved to be the highest in several organs of grown-up animals and human [8]. Our previous study showed that the body and tail of mouse and human sperm also expressed high levels of NR2B [9]. Despite extensive study of NR2B, its biological properties and ligand bioactivities in spermatogenic system remain poorly understood. This study aimed to determine whether NR2B-targeting compounds could affect sperm activity. MK801, which was adopted as a candidate compound of NR2B antagonists due to its higher potency, was used to treat human sperm; a seminal fluid analyzer was applied to monitor the change of sperm movement after the drug intervention; and also, a cervix-mucilage interaction experiment was utilized simultaneously to explore the influence of MK801 on the fertilizing capacity of sperm.

## Materials and Methods

### Detection of Sperm Activity

MK801 was bought from Sigma Company. Human sperm were obtained from several 25~40-year-old men with a marriage and child-rearing history. All sperm samples were obtained via masturbation. On experimentation, sperm were suspended with BWW fluid [10] (BWW stock solution: To 1000 ml of purified water added 5.54 g of sodium chloride (NaCl), 0.356 g of potassium chloride (KCl), 0.294 g of magnesium sulfate heptahydrate (MgSO<sub>4</sub>·7H<sub>2</sub>O), 0.250 g of calcium chloride dehydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O) and 0.162 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>). Then, adjust the pH to 7.4 with 1 mol/l sodium hydroxide (NaOH), add 1.0 ml (0.04%, 0.4 g/l) phenol red per litre. On the day of use: Supplement 100 ml of stock solution with 210 mg of sodium bicarbonate (NaHCO<sub>3</sub>), 100 mg of D-glucose, 0.37 ml of 60% (v/v) sodium lactate syrup, 3 mg of sodium pyruvate, 350 mg of fraction V bovine serum albumin, 10 000 units of penicillin and 10 mg of streptomycin sulfate, warm to 37 °C before use in an atmosphere of 5% (v/v) CO<sub>2</sub>, 95% (v/v) air.) In a water bath at 37°C after liquation, and then adjusted to 6×10<sup>7</sup>/ml. The specimen were treated with MK-801 at different concentrations and subsequently incubated in a water bath at 37°C for 30 min. Finally, motility measurement was performed using a US Hamilton-IVOS sperm analyzer (computer assisted sperm analysis system, CASA). The process was repeated 3 times. No less than 1×10<sup>3</sup> spermatozoa were analyzed per treatment. Every treatment was repeated for 5 times. All the sperm tested from each treatment were from the same sample.

### Sperm-Cervical Mucus Contact (Scmc) Test

Samples of cervical mucus were prepared by using an absorption method. The cervical-mucus preparation was conducted 1~2 days prior to ovulation. The standards of performance for cervical mucus collection were that each supplier was required to be a healthy female at the age of 25-40 years old with a marriage and child rearing history and that, according to the Moghissi scoring system, sample with a score higher than 10 was considered favorable. Preparation and treatment of sperm specimen were done as mentioned above. A sperm-cervical mucus contacting test was initiated by addition of the MK-801-pretreated sperm to the cervical mucus system. According to the reference [10], SCMC test was preformed. The experiment was repeated 3 times. All the sperm tested from each treatment were from the same sample.

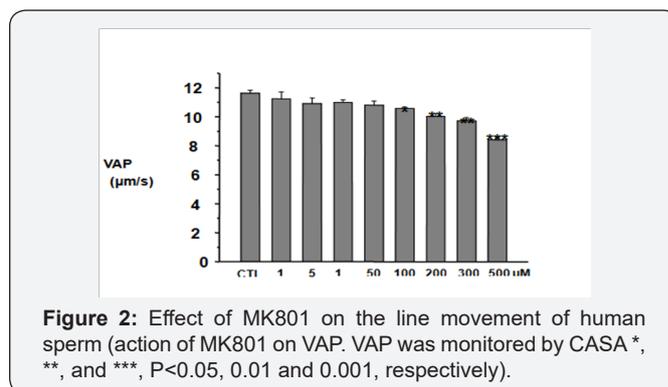
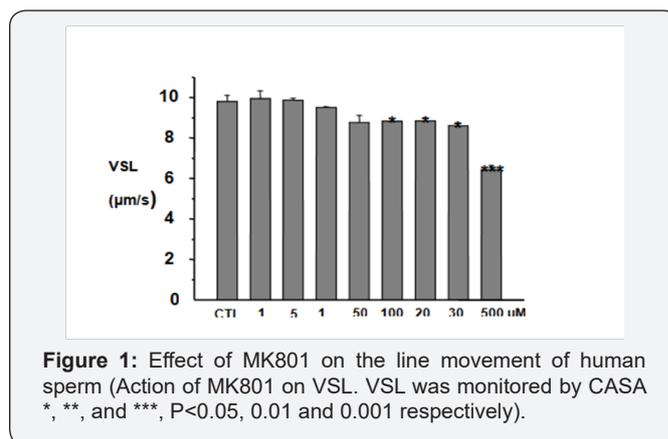
### Statistical analysis

Data were expressed as mean ± standard deviation for all continuous variables. One-way analysis of variance (ANOVA) was used for comparing experimental data. P values less than 0.05 were considered to be statistically significant. Statistical analysis was performed using the statistical software Origin 7.0 (Origin Lab, Northampton, MA).

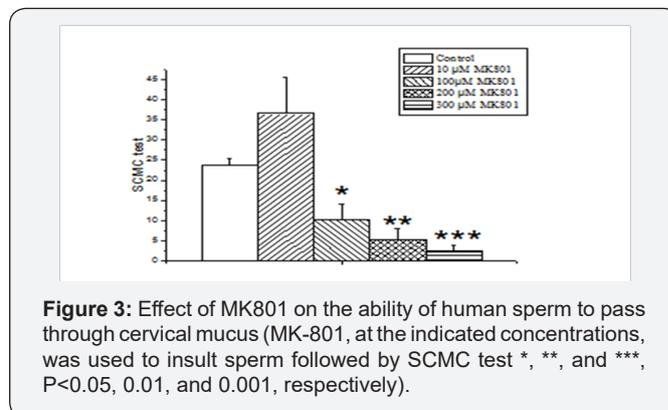
## Results

### Effect of Mk-801 on Human Sperm Movement

As shown in Figure 1, straight-line velocity (VSL) and average path velocity (VAP) of sperm movement were significantly decreased after treatment of sperm with 1µm, 5µm, 10µm, 50µm, 100µm, 200µm, 300µm and 500µm MK-801, with P value less than 0.05 as compared to control group, demonstrating that the direction and speed of sperm movement were disturbed by MK-801 (Figures 1 & 2).



### Effect of Mk-801 on Sperm Penetration of Cervical Mucus



After the MK-801-pretreated sperm and the cervical mucus were mixed together, passage of the sperm through cervical

mucus was measured by using a SCMC test. As shown in Figure 2, groups in which the sperm had experienced an early incubation with concentrations of MK-801 prior to incorporation showed lower levels of SCMC values, relative to control group with no MK-801 pre-incubation (each  $P < 0.05$  for group with 100, 200, or 300  $\mu\text{m}$  MK-801), illustrating that MK-801 inhibited the ability of sperm to pass through cervical mucus (Figure 3).

### Discussion

It is reported that  $\gamma$ -aminobutyric acid (GABA) system exists in the testicle tissue and sperm of rats and mouse and has a vital impact on the maturation of sperm [11-14]. The present study is an extension of our early investigation that disclosed that NR2b was highly expressed in the body and tail of mice and human sperm [9]. This high expression provides an indication that NR2B inhibitors may affect the biological properties of human sperm. When using MK-801, a potent selective, non-competitive antagonist of NMDA receptors, to treat human sperm, it was shown that the VSL and VAP of sperm movement decreased significantly in the groups with 100 and 500  $\mu\text{m}$  of the testing compound. Indeed, MK-801 influenced the physiological event of sperm. This phenomenon suggests that MK-801 could interfere with sperm-ovum interactions. To test this hypothesis, an in vitro SCMC test was conducted. Obviously, MK-801, in a concentration-dependent manner, strongly inhibited the sperm penetration capacity at the contact interface between sperm and cervical mucus. Our current finding pharmacologically confirms the NR2B-targeting hypothesis in sperm-ovum interactions. The capacitating procession occurs in female uterus. After maturation in epididymis, large quantity of decapacitation factor exists in seminal plasma. The majority of decapacitation factor can be blocked by cervical mucus after sexual intercourse. However, the effect of MK801 in present study was to block the NR2B receptor, disturb the motility of spermatozoa, rather than hamper the procedure of sperm capacitating. Thus, it is no conflict to the spermatozoa capacitating process in present study after using BWB solution as an alternative for seminal plasma.

Recently, several external contraceptives such as alfenoxynol and benzene alcohol in different formulations are extensively used in clinic. Generally speaking, the currently used contraceptives or compounds with clinical potential mainly act by killing sperm. In this study, the glutamate receptor NR2B highly expressed in human sperm was adopted as a therapy target, and its potent selective antagonist MK-801 was used to assess the possible pharmacological actions. As expected, our

results showed that this NR2B-targeting compound was able to produce motility-inhibitory activities as mentioned above. NMDA receptor antagonists such as MK-801 are thereby suggested to have the potential for contraceptive use. In summary, NMDA receptor antagonist MK801 reduces the linear activity of human sperm and impairs sperm penetration through cervical mucus. Results from this study will provide a new idea for solving the problems of human reproduction and contraception.

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