EFFECTS OF ANGIOTENSINS ON THE MOTILITY OF HUMAN SPERM

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The effects of angiotensins on the motility of highly washed human sperm were examined by multiple exposure photography system. The addition of low doses (0.1–10 ng/ml) of angiotensin II (AT II) stimulated the forward velocity of sperm without increasing the percentage of motility. On the contrary, high concentration (1.0 μ g/ml) of AT II suppressed the motility.

The direct effect of angiotensin I (AT I) on the motility was observed under the presence of SQ 14225, a specific inhibitor of angiotensin I converting enzyme (ACE). AT I (0.1–100 ng/ml) gave no influence on the motility, whereas that of high dose (1.0 μ g/ml) suppressed the motility in the same manner as the corresponding dose of AT II.

The action of AT II on the motility was completely suppressed under the coexistence of saralasin, a specific antagonist against AT II. This fact indicated that the action of AT II on the sperm appeared through the specific receptor on sperm.

Keyword — sperm; motility; angiotensin I; angiotensin II; saralasin; SQ 14225; angiotensin I converting enzyme

INTRODUCTION

Dipeptidyl carboxypeptidase (DCP, EC 3.4.15.1) is a component of kallikrein-kinin and renin-angiotensin systems and mediated both the kinin degradation and the conversion of AT I to AT II.¹⁾ DCP has been found in the blood, kidney and lung of various mammals.¹⁾ The present authors reported previously that seminal plasma and testis of various mammals had high DCP activities and that the activity in the rat testis was increased with sexual maturation, and this increment was suppressed by treatment of danazol,²⁾ an anti-FSH, LH agent.³⁾ We also found that DCP activity was significantly lower in the semen of oligo- or asthenospermic men than that in normal men.³⁾ The motility of human sperm was stimulated by bradykinin and kallidin, the physiological substrates of DCP.⁴⁾ These observations led to the supposition that kinins and DCP might be related to the male reproductive functions.

We report here that angiotensin, another

physiological substrate of DCP (ACE), also stimulates the motility of human sperm.

MATERIALS AND METHODS

Human Sperm — The ejaculates of healthy donors were obtained after 4 d sexual abstinence and their sperm density and the percentage of motility were measured subjectively.

Sperm Culture Medium — The medium for sperm culture was composed of 20 mM HEPES, 138 mM NaCl, 10 mM glucose, 2 mM Na₂HPO₄, 4 mM MgCl₂, 2.5 mM KOH and 7.5 mM NaOH. The medium was given at pH 7.5 with 290–300 m osmole by freezing point depression.

Washing Procedure of Sperm⁵⁾ — Liquified ejaculates from several donors were combined and diluted twice with the culture medium. The diluted semen was carefully layered on 7.5 % (w/v) Ficoll in the culture medium, then the tube was centrifuged in a swing-out rotor at 250 $\times g$ for 10 min. After centrifugation, the Ficoll

layer and the seminal plasma which remained on the top were removed by aspiration and the loose pellet of sperm was resuspended in the culture medium and made up to 5×10^7 sperm/ml.

Quantitative Assessment of Sperm Motility

—To analyze the percentage of motility and the velocity of motile sperm quantitatively, a multiple exposure photography method⁵⁾ was adopted in this investigation.

A drop of incubated sperm suspension (6.0 μ l) was placed on a glass slide and covered with a coverslip. The thickness of sperm suspension was about 15 μ m. The glass slide was put on the microscope stage (magnification: 10×40) which was kept at 37°C with the pyrostat. The image was photographed stroboscopically 3 times with 1 s intervals, and the micrometer was also photographed simultaneously. The developed film was projected on paper, and the tracks of forward motile sperm in 2 s were recorded as lines. Submotile and immotile sperm were marked as triangles and dots, respectively. The recorded tracks were traced with a curve meter, and converted to μ m/s. The percentage of motility and the average velocity of forward motile sperm were calculated by the following equations, and the numerical value in the figure was the average of 3 pictures in each specimen.

Equations : The average velocity of forward motile sperm (μ m/s) = (total distance of recorded lines/No. of forward motile sperm) $\times$ 1/2

The percentage of motile sperm = (No. of forward motile and submotile sperm/No. of sperm in the view area) $\times$ 100

Determination of Angiotensinase and ACE Activities — Angiotensinase activity was measured by bioassay on an isolated rat uterus according to a modification of the method described in the previous report.³⁾ The washed sperm suspension (100 μ l) was incubated with 10 ng AT II (100 ng/ml) for 30 min at 30°C. The reaction mixture was poured on a rat uterus segment. The contractile response caused by the AT II remaining in the mixture was compared with that caused by 10 ng AT II.

The inhibition of ACE activity by SQ 14225⁶⁾

was also estimated with a rat uterus. Equal volumes of the washed sperm suspension and various concentrations of SQ 11225 (5.34–534 μ g/ml) were mixed and preincubated for 5 min at 30°C. Then 10 ng of AT I (100 ng/ml) was added to each mixture (100 μ l) and they were further incubated for 30 min at 30°C. The contractile response by AT II converted from AT I was compared with that induced by 10 ng AT II.

Chemicals — Ficoll 400 was the product of Pharmacia Fine Chemicals (Uppsala, Sweden). Angiotensin I (AT I), angiotensin II (AT II) and saralasin (1-Sar-8-Ala-AT II) were obtained from Protein Research Foundation (Osaka, Japan). SQ 14225 (D-3-mercapto-2-methylpropanoyl-L-proline) and HEPES (N-2-hydroxyethylpiperadine-N-2-ethane sulfonic acid) were the products of Sankyo Co. (Tokyo, Japan) and Nakarai Chemicals Co. (Tokyo, Japan), respectively.

RESULTS

Detection of Angiotensinase Activity in Human Semen

The presence of angiotensin degradation activity (angiotensinase) in the human seminal plasma disturbed the estimation of the net effects of angiotensins. This activity was measured with the isolated rat uterus.

Even after incubation for 30 min, the contractile response caused by AT II remaining in the mixture was almost identical with that by 10 ng AT II. Therefore, there was little angiotensinase activity in the washed human sperm suspension and its influence could be neglected in the present investigation.

Protection of AT I from the Residual ACE during Incubation

As described previously, even after washing there was still a little contamination of ACE (2–3% of the original semen) in the sperm resuspension.³⁾ When AT I was incubated with the washed sperm suspension, it was gradually converted to AT II by the residual ACE. To estimate the direct action of AT I, ACE activity was inhibited completely without harmful influence

on the sperm physiology. For this purpose, SQ 14225,⁶⁾ a specific and potent inhibitor against ACE, was adopted in this investigation.

The rat uterus contraction caused by the converted AT II completely disappeared in the presence of more than 2.17 $\mu\text{g/ml}$ of SQ 14225. On the basis of these results, the effect of AT I was observed in the presence or absence of 2.17 $\mu\text{g/ml}$ of SQ 14225.

Effect of Angiotensin II on the Motility of Washed Human Sperm

As shown in Fig. 1, the addition of 0.1–10 ng/ml AT II accelerated the velocity of forward motile sperm, but not so increasing the percentage of motility. The effect was remarkable between 0.5 and 2 h incubation, the fastest velocity being marked at 0.5 h. On the contrary, the higher dose of AT II (1.0 $\mu\text{g/ml}$) suppressed the motility through the decrease in both of motility indices. Finally, all sperm in the field of view became immotile after 4 h.

Effect of Angiotensin I on the Motility of Washed Human Sperm

The direct action of AT I on sperm motility was estimated by the aid of SQ 14225, and the presence of the agent had no influence on the motility (Fig. 2). As described above, the addition of AT II (0.1–10 ng/ml) induced the acceleration of the velocity of forward motile sperm (Fig. 1), whereas these doses of AT I which were kept intact by SQ 14225 gave no influence on both of motility indices. On the other hand, the absence of SQ 14225 allowed the conversion of AT I to AT II, and sperm motility was increased in the same manner as the corresponding doses of AT II. Although low doses of AT I were ineffective on sperm motility, that of high dose (1.0 $\mu\text{g/ml}$) suppressed both the velocity of forward motile sperm and the percentage of motility as with AT II.

The Influence of Saralasin on the Effect of Angiotensin II

In order to clarify the manner of AT II action on the sperm in more detail, the effect of AT II was examined in the presence of saralasin,⁷⁾ a specific antagonist against AT II. All the doses of

saralasin examined (final concentration 0.1–100 ng/ml) had no influence on the motility. It decreased, however, the velocity of forward motile sperm to the level of the control even in the presence of 1.0 ng/ml of AT II. Only the results obtained with 0.1 ng/ml saralasin were shown in Fig. 3. The action of AT II on the sperm motility was completely antagonized by 0.1 ng/ml of saralasin. This fact indicated that the action of AT II on the sperm was exerted through the specific receptor on the sperm.

DISCUSSION

Although human seminal plasma contains a variety of substances which may affect sperm physiology, the investigations on sperm have usually been conducted merely with diluted semen to avoid any injury during centrifugation for washing. The existence of ACE in the seminal plasma³⁾ was not neglected to evaluate the direct action of AT I on sperm. Thus we adopted a washing procedure with Ficoll⁵⁾ by which 97% of the seminal plasma could be eliminated without loss of sperm motility. Furthermore the residual ACE was completely inhibited by SQ 14225, which is a newly synthesized thiol derivative of proline and a highly specific inhibitor for ACE.⁶⁾ It was confirmed that SQ 14225 itself had no effect on sperm motility as shown in Fig. 2. It was also shown that another noticeable contaminant, angiotensinase, was negligible in the washed human sperm suspension.

As described in the previous report,⁴⁾ bradykinin and kallidin caused obvious stimulation on human sperm motility. This phenomenon was observed with rather small doses (0.1–10 ng/ml) of these substances and was manifested as an acceleration of the forward velocity, but not as an increase in the percentage of motility, whereas at the higher dosage (1.0 $\mu\text{g/ml}$) the motility was suppressed. The mode of action and the dose response relationship of AT II on sperm motility were very similar to those of kinins.

Angiotensins and kinins are the active principals of renin-angiotensin and kallikrein-kinin systems, respectively. These systems have

FIG. 1. *Effect of Angiotensin II on the Forward Velocity and the Percentage of Motility of Washed Human Sperm*

Equal volumes of the washed sperm suspension and various concentrations of AT II ($0-2 \times 10^3$ ng/ml of the culture medium) were mixed and cultured at 22°C , and sperm motility was examined for 4 h.

○ : control, ● : angiotensin II.

FIG. 2. Effect of Angiotensin I on the Forward Velocity and the Percentage of Motility of Washed Human Sperm

The effect of AT I on the sperm motility was observed in the presence or absence of 5.34 ng/ml of SQ 14225. The washed sperm suspensions (0.3 ml) were preincubated for 5 min at 22°C with 0.1 ml of either the culture medium or 26.7 ng/ml of SQ 14225, then the preincubation mixtures were cultured at 22°C for 4 h with 0.1 ml of various concentrations of AT I (0–5.0 μg/ml). The sperm motility was observed hourly in the same manner as that of AT II.

---○---: control, —○—: SQ 14225 (5.34 ng/ml), ---●---: angiotensin I, —●—: angiotensin I + SQ 14225 (5.34 ng/ml).

FIG. 3. Influence of Saralasin on the Stimulating Action of Angiotensin II on the Motility of Washed Sperm

The washed sperm suspensions (0.3 ml) were preincubated for 10 min at 22°C with 0.1 ml of saralasin (0, 0.5, 5.0, 50 and 500 ng/ml in the culture medium), then 5 ng AT II (50 ng/ml in the culture medium) was added to each mixture and they were cultured in the same manner as the case of AT II.

---○--- : control, ---●--- : angiotensin II (1.0 ng/ml), —○— : saralasin (0.1 ng/ml), —●— : angiotensin II (1.0 ng/ml) + saralasin (0.1 ng/ml).

complementary action in the regulation of blood pressure or salt metabolism, *etc.*⁸⁾ The direct effect of these principals are usually revealed on the contraction of smooth muscles. In the case of sperm they would also affect the motility in a similar mode of action.

AT I was considered as the poor or inactive precursor of AT II. It was confirmed by the aid of SQ 14225 that the low doses of AT I were inactive on sperm motility, although AT I itself strongly suppressed the motility in high dose as with AT II. Fujio *et al.* revealed the presence of AT I and AT II in human semen using the radioimmunoassay, and the concentration of AT II was found to be 35.3 ± 1.6 pg/ml.⁹⁾ Therefore, AT II might act physiologically as a stimulant of sperm motility, and AT I in semen might exert its activity after conversion to AT II, but not itself.

Saralasin (1-Sar-8-Ala-AT II) was synthesized as an antagonistic analogue against AT II.⁷⁾

Regolu *et al.*¹⁰⁾ studied the influence of 1-Sar-8-Leu-AT II, a familiar type of saralasin analogue, for the action of AT II on smooth muscle, and suggested that the agent irreversibly occupied a fraction of AT II receptors, and acted as a non-competitive antagonist against AT II. In the present investigation, it could be considered that the suppression of AT II action by saralasin was also exerted through antagonism against AT II receptors. In turn, this fact indicated the existence of AT II receptors in the sperm.

There have been few reports discussing the significance of the local hormones such as kinins and angiotensins on sperm physiology. During a series of our studies, it was found that kinins⁴⁾ and AT II affected sperm motility, and the activity of ACE in the human semen was positively correlated to semen qualities such as sperm motility or density, even though ACE itself did not affect sperm motility.³⁾

It is considered that the contraction and relax-

ation of the uterus contribute to the sperm transportation through the female genital tract. Kinins and AT II contract the uterus, and the forward velocity of motile sperm is also accelerated by low doses of these substances. These facts led to the supposition that the local hormone systems such as kallikrein-kinin and renin-angiotensin systems might be related to sperm physiology and fertilization.

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