精巣上体通過に伴うラット頭部後域のトリプシン抵抗性の増大

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	松沢, 時弘 Hamilton, D.W.
巻/号	49巻4号
掲載ページ	p. 741-744
発行年月	1987年8月

Increase in Trypsin Resistance of the Posterior Portion of the Rat Sperm Head Upon Entering the Epididymis[1)]

Tokihiro MATSUZAWA, and David. W. HAMILTON[2)]
Laboratory of Biology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro 080, and [2)]Department of Cell Biology and Neuroanatomy, University of Minnesota, 4–135 Jackson Hall, 321 Church Street, SE Minneapolis, MN 55455, U. S. A.
(Received 21 July 1986/Accepted 12 May 1987)

Jpn. J. Vet. Sci. 49(4): 741–744, 1987

KEY WORDS: epididymal transit, posterior head, trypsin digestion.

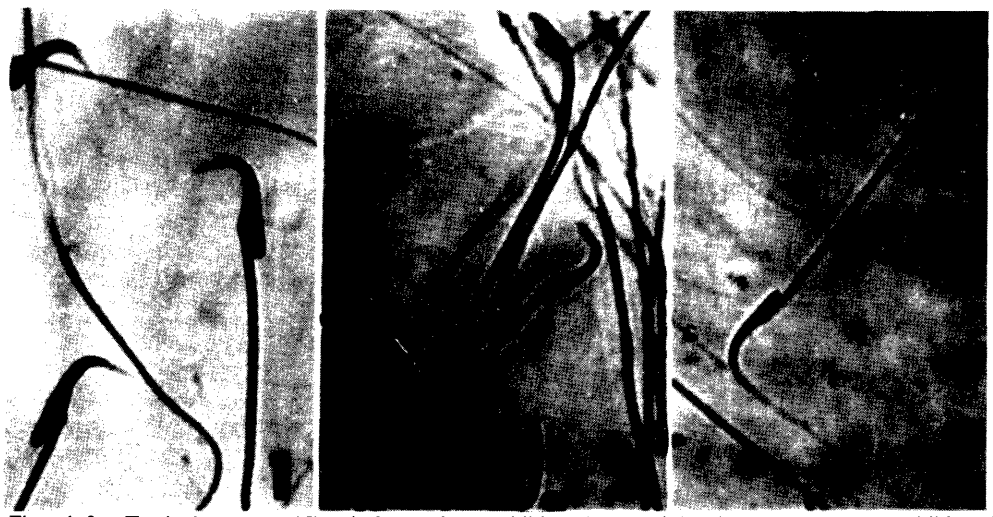
In mammals a series of morphological and biochemical changes take place in the sperm during its epididymal transit [1]. These changes are prominent on the surface of cell; for example, in adhesive characteristics [4], and in the specific surface saccharide residues as shown by lectin binding properties [7, 14] and biochemical labeling of cell surface carbohydrates [8]. Based on morphological evidence from freeze fracture analysis, the sperm plasma membrane differs markedly between the tail (midpiece) and the head (acrosomal and post-acrosomal region) [12, 13].

The present report describes the effects of digestion with trypsin and alpha-cymotrypsin on the posterior portion of sperm head in the rat.

Adult Wistar rats (80–90 day old) were used. Testicular sperm were obtained by centrifugation from rete testiculalr contents which were collected by the method of Hamilton [5]. After the epididymis was cut into 3 segments (caput, corpus, and cauda), sperm was squeezed out from each segment using forceps. It was suspended in the buffered saline (PBS; pH 7.4) and was washed three times with the PBS, and spread out on a cover slip at room temperature till it dried. This preparation was then treated with 30 units per m*l* of trypsin or alpha-chymotrypsin at 37°C in PBS. After the enzymatic treatment some preparations of the sperm were additionally treated with DNase (30 units/m*l*) for 10 minutes at 37°C. Both intact and enzyme treated sperm on the coverslips were stained without fixation with toluidine blue for morphological observation [10]. Trypsin, alpha-chyomtrypsin and DNase were purchased from Sigma Chemical Company, Inc. (St. Louis, Missouri).

In intact sperm the intensity of staining of the head with toluidine blue decreased as sperm passed through the epididymis. In cauda sperm no staining reaction was observed (Fig. 1, 2, 3).

After treatment with trypsin for 30 minutes, the posterior portion of sperm head in 97% of the



Figs. 1–3. Testicular sperm, (Fig. 1), Sperm from epididymal caput, (Fig. 2), and Sperm from epididymal cauda (Fig. 3). ×600

testicular sperm became partially digested or swollen. The swollen head showed a more intense staining reaction with toluidine blue. Only 3% of testicular sperm were unaffected by the trypsin treatment. The staining intensity in most of the testicular sperm was unchanged between 30 min and 1 hr period of the enzyme treatment. Some of the sperm lacked the posterior portion of the head because they had been completely digested (Fig. 6). In testicular sperm treated with alpha-chymotrypsin for 30 min or 1 hr, an intense stain was observed in a restricted area of the dorsal part of the posterior head (Fig. 7). In contrast, more than 95% of caput and cauda epididymal sperm showed resistance to trypsin digestion (Fig. 5). Epididymal sperm was more resistant to alpha-chymotrypsin than testicular sperm. These data are summarized in Table 1 and 2.

The intense stain was not affected by DNase digestion which was followed by trypsin or alpha-chymotrypsin treatment. After sperm was fixed with 4% glutaraldehyde, the trypsin or alpha-chymotrypsin treatment did not enhance the intensity of toluidin blue stain.

Calvin and Bedford [2] have indicated that major stabilization of the nuclear chromatin dependeds on the formation of disulfide cross-links during sperm maturation in the epididymis, and that establishment of the disulfide bonds occurs also in the sperm tail during this period. Olson and Hamilton [8] reported that the perforatorium of rat sperm became more resistant to detergent solubilization during epididymal transit. In the present invesitgation the posterior portion of the head also becomes more resistant to enzyme digestion during the transit.

The activity of γ-glutamyltranspeptidase (γ-GTP), important in the synthesis and digestion of glutathione via the γ-glutamyl cycle, is the

Fig. 4. Testicular sperm treated with trypsin for 30 min. (×600)

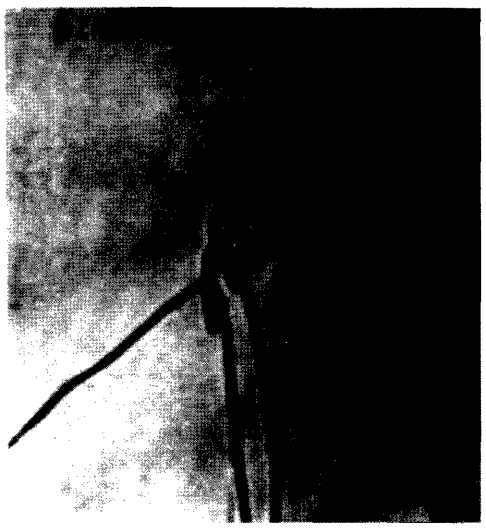


Fig. 5. Caput sperm treated with trypsin for 30 min. (×600)

Table 1. Effect of trypsin treatment on the posterior portion of sperm head

	30 min.		60 min.	
	Not affected	Swollen or Digested	Not affected	Swollen or Digested
Testicular sperm	7 (3.0%)[a)]	229 (97.0%)	0 (0 %)	182 (100 %)
Caput sperm	200 (95.7%)	9 (4.3%)	205 (95.3%)	10 (4.7%)
Cauda sperm	189 (95.9%)	8 (4.1%)	181 (95.2%)	9 (4.8%)

a) Number of cells observed and frequency.

Table 2. Effect of α-chymotrypsin treatment on the posterior portion of sperm head

	30 min.		60 min.	
	Not affected	Deeply stained or Partially digested	Not affected	Deeply stained or Partially digested
Testicular sperm	17 (8.3%)[a)]	188 (91.7%)	0 (0 %)	135 (100 %)
Caput sperm	—	—	201 (95.7%)	9 (4.3%)
Cauda sperm	115 (95.8%)	5 (4.2%)	106 (96.4%)	4 (3.6%)

a) Number of cells observed and frequency.

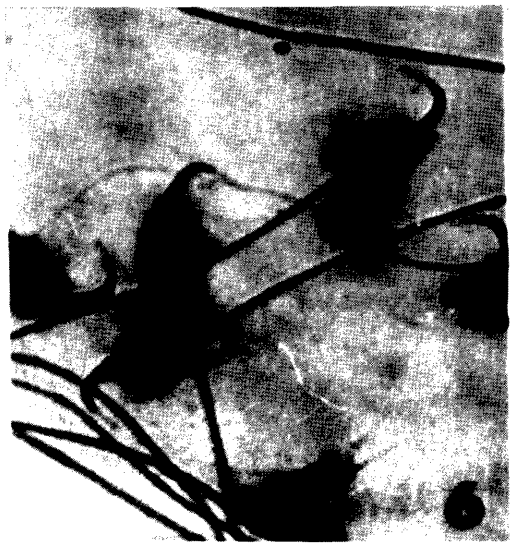


Fig. 6. Testicular sperm treated with trypsin for 1 hr. (×600)

Fig. 7. Testicular sperm treated with alpha-chymotrypsin for 30 min. (×600). Arrows indicate the dorsal region in the posterior sperm head showing the intense reaction.

highest in the caput of rat epididymis [6]. Glutathione-S-transferase, which makes disulfide bonds from sulfhydril residues, is higher in activity in the caput than the cauda [11]. The increase in trypsin-resistance which the posterior portion of the sperm head aquires may depend on an increase in disulfide bonds and sterically inhibitan access of the protease to the surface proteins.

Trypsin attacks arginine- and lysine-residues in polypeptides, whereas alpha-chymotrypsin has affinity for residues of tyrosine, tryptophane, phenylalanine and leucine [3]. In the present investigation the area attacked by alpha-chymotrypsin was limited to small dorsal area of the posterior portion in sperm head, while trypsin attacked the whole posterior portion. This suggests that the surface proteins of posterior portion of rat sperm head may have some regional specificity.

Enhanced staining reaction induced by the treatment with trypsin or alpha-chymotrypsin may result from denaturing of surface proteins but not of nucleoprotein, because DNase treatment did not affect the intensity of the staining.

ACKNOWLEDGEMENT. This research was supported by USPHS grant HD-11962 and grant from the Andrew W. Mellon Foundation to DWH.

REFERENCES

1. Bedford, J. M. 1975. pp.303–317. In: Handbook of Physiology, vol. 5, Sec. 7 (Hamilton, D. W. and Greep, R., ed.), American Physiological Society, Washington, D. C.
2. Calvin, H., and Bedford, J. M. 1971. *J. Reprod.*

Fertil. (Suppl.) 13: 65–75.
3. Dixon, M., and Webb. E. L. 1979. pp.884–886. In: The enzymes (3rd ed.), Longman Group Ltd., London.
4. Fawcett, D. W., and Hollenberg, R. D. 1963. *Z. Zellforsch.* 60: 276–292.
5. Hamilton, D. W. 1980. *Biol. Reprod.* 23: 377–385.
6. Matsuzawa, T. 1981. *Endocrinol. Japon.* 28: 469–475.
7. Nicolson, G. L., Usui, N., Yanagimachi, R., Yanagimachi, H., and Smith, J. R. 1977. *J. Cell Biol.* 74: 950–962.
8. Olson, G. E., and Hamilton, D. W. 1978. *Biol. Reprod.* 19: 26–35.
9. Olson, G. E., Hamilton, D. W., and Fawcett, D. W. 1976. *J. Reprod, Fertil.* 47:294–297.
10. Pearse, A. G. E. 1985. pp.742. In: Histochemistry, vol 2 (4th ed.). Churchill Livingstone, Edinburgh, London, Melbourune, and New York.
11. Robaire, B., and Hales, B. F. 1982. *Biol. Reprod.* 26: 559–565.
12. Suzuki, F. 1981. *Anat. Rec.* 199: 361–376.
13. Suzuki, F., and Nagano, T. 1980. *Biol. Reprod.* 22: 1219–1231.
14. Temple-Smith, P. D., and Bedford, J. M. 1976. *Am. J. Anat.* 471–500.

要　約

精巣上体通過に伴うラット頭部後域のトリプシン抵抗性の増大（短報）：松沢時弘・Hamilton, D. W.[1]（帯広畜産大学・一般教育課程生物学研究室，[1]Department of Cell Biology and Neuroanatomy, University of Minnesota, U. S. A）——精巣上体を通過する間にラット精子の頭部後域のトリプシン，α-キモトリプシンに対する抵抗性が増大した。精巣精子では，α-キモトリプシンによって消化される頭部後域がその一部に限局されていたのに対し，トリプシンの場合は，頭部後域全体が消化された。