MEIOTIC DRIVE IN t^n -BEARING MOUSE SPERMATOZOA: A RELATIONSHIP BETWEEN AEROBIC RESPIRATION AND TRANSMISSION FREQUENCY

L. GINSBERG AND NINA HILLMAN

Department of Biology, Temple University, Philadelphia, Pa. 19122, U.S.A.

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Summary. The present studies show that the higher transmission ratio of mutant t^n spermatozoa in mice can be correlated with a reduction in the NADH/NAD ratio and increased oxygen uptake, both reflecting an increased rate of energy metabolism. It has been shown that the increased energy metabolism is related to the presence of the t^n allele in the pooled sample; therefore, it is postulated that the meiotic drive associated with T^+/t^n mice is a function of the increased energy metabolism of the t^n -bearing spermatozoa.

INTRODUCTION

Meiotic drive has been defined as either an alteration of normal meiosis (Sandler & Novitski, 1957) or as a transmissional anomaly (Lewontin, 1962) which results in an increased transmission frequency of spermatozoa carrying one gene over those carrying its allele. The increased transmission rate is independent of any deleterious effect of the driven gene. One example of meiotic drive is found in mice, where certain t^n alleles of the T (or *brachyury*) locus are transmitted in a non-Mendelian frequency (Dunn, 1960; Yanagisawa, Dunn & Bennett, 1961). The dominant short-tail allele, T , is an embryonic lethal in the homozygous condition (Dunn, 1956), but in combination with other mutant alleles (T/t^n) or with its wild-type allele (T^+/T) produces viable offspring which are tailless or short-tailed respectively. Males heterozygous for the dominant short-tail allele (T^+/T) transmit the alleles in a 1:1 ratio, as do also all heterozygous females (T^+/T ; T/t^n). Conversely, males heterozygous (T^+/t^n ; T/t^n) for certain t^n alleles transmit the t^n allele with a frequency greater than 50%. There is no evidence that the male segregation ratio abnormality is the result of extra postmeiotic mitoses of t -allele-bearing spermatozoa or that these spermatozoa are present in significantly higher numbers in seminal fluid (Bryson, 1944).

It has been suggested that t^n -bearing spermatozoa have a physiological advantage over T^+ or T spermatozoa and that the time of this advantage is between ejaculation and fertilization (Braden, 1958). We are proposing that increased motility, capacitation and/or fertilization could yield this advantage.

Since the levels of these functions have been shown to be directly proportional to the levels of aerobic metabolism in mammalian spermatozoa (Hamner & Williams, 1963), an investigation was begun to determine the levels of respiration of T^+ , T , and t^n epididymal spermatozoa. A preliminary study suggested that there were energy differences between pooled spermatozoa from T^+/T^+ , T^+/T , and T^+/t^n males (Ginsberg & Hillman, 1973). We have continued this work to elucidate further the connection between meiotic drive and atypical levels of aerobic respiration by t^n -bearing spermatozoa.

MATERIALS AND METHODS

Male mice used in these experiments were either T^+/T , T^+/t^{w32} , T^+/t^{12} , T^+/t^6 or T^+/T^+ . The heterozygous mutant males were obtained by mating T/t^{w32} , T/t^6 or T/t^{12} males to $BALB/cT^+$ homozygous females, producing phenotypically normal-tailed (T^+/t^n) or short-tailed (T^+/T) offspring. The t^{w32} allele was placed on a $BALB/c$ background and back-crossed to $BALB/c$ for five generations, yielding both control $BALB/cT^+ / BALB/cT^+$ and experimental $BALB/cT^+ / BALB/ct^{w32}$ males. Additional control spermatozoa were obtained from randomly bred Swiss Albino mice and $BALB/c$ mice which had been inbred for forty-seven generations. Using epididymal spermatozoa, two energy metabolism parameters were studied: comparative NADH/NAD ratios and O_2 uptake (ZO_2) in the presence of specific substrates. The frequencies of transmission of the t^{12} and t^{w32} alleles are similar (Smith, 1956; Ginsberg & Hillman, 1973) and our data show that the NADH/NAD ratios and O_2 uptake by pooled spermatozoa from both T^+/t^{12} and T^+/t^{w32} males are not significantly different. Data are presented, therefore, only for T^+/t^6 and T^+/t^{w32} genotypes.

NADH/NAD ratios

Epididymal spermatozoa were collected in 300 μ l cold calcium-free, PO_4 -buffered sperm Ringer (pH 7.4). A 50- μ l sample was used for density reading, and two 100- μ l aliquots were removed for separate extractions and determinations of NAD and NADH (Glock & McLean, 1955). One 100- μ l aliquot was added to 200 μ l of 0.1 N-HCl (to extract NAD) at 85°C, and the other to 200 μ l of 0.1 N-NaOH (to extract NADH) at 85°C. The sperm suspensions were incubated at 85°C for 2 min, immersed in an ice bath for an additional 3 min and the suspension was then neutralized with either 0.1 N-NaOH or 0.1 N-HCl. The concentrations of NAD and NADH were determined by an oxygen electrode at 25°C (Greenbaum, Clark & McLean, 1965). The density of the 50- μ l aliquot was read spectrophotometrically at 500 nm and the number of spermatozoa determined by comparing the sample density to standard curves of known numbers of spermatozoa (Nelson, 1972). These density readings are correlated directly with the number of spermatozoa and are independent of the genotype. Each mutant sample contained two types of spermatozoa, wild-type and mutant, whereas control samples were composed entirely of wild-type spermatozoa.

O₂ uptake

The protocol for the O₂ uptake studies was a modification of that described by Peterson & Freund (1970). A 50- μ l aliquot of epididymal spermatozoa was diluted to 1 ml with a modified tris-based salt medium, pH 7.4, supplemented with 10 mM of specific substrate (glucose, fructose, succinate, lactate, pyruvate). The sperm suspension was placed in an oxygen electrode vessel at 37°C, stirred and allowed to equilibrate for 5 min. The electrode was inserted and O₂ uptake was recorded for 3 to 5 min. A 50- μ l sample was removed and the number of spermatozoa was determined spectrophotometrically.

RESULTS

Table 1 shows the inverse relationship between the NADH/NAD ratios and the frequency of sperm transmission. The transmission frequency of T^+/T^+ and T^+/T males was 1:1, and in pooled sperm samples from both of these genotypes the NADH/NAD ratio was approximately 0.8 (similar to the NADH/NAD ratio reported for other mammalian spermatozoa by Brooks & Mann, 1971). In the pooled spermatozoa from T^+/t^n males, however, the NADH/NAD ratios were lower, with the T^+/t^6 sample having the lowest NADH/NAD ratio. The lowered ratio in spermatozoa from recessive heterozygotes was, in each case, a result of lower NADH and higher NAD concentrations. These results reflect an increased rate of energy metabolism in the pooled spermatozoa containing the t^n mutant. While consistent differences were found between genotypes, no significant differences were noted in spermatozoa taken from males of different ages (12 weeks to 1 year) but of the same genotype.

It can be seen from Table 2 that both T^+/T^+ and T^+/T samples utilized substrates equally, whereas the t^n -containing samples showed a ZO₂ increase over comparable T^+/T^+ and T^+/T samples in the presence of fructose, lactate, pyruvate and succinate. The data show that the percentage difference between either the recessive mutant-containing pooled sample and the T^+/T^+ and T^+/T samples increased with the proximity of the substrate to the TCA cycle. Only with glucose did the T^+/T^+ and T^+/T samples have a higher ZO₂ uptake than the recessive mutant-containing samples. In this series of experiments, the spermatozoa from the progeny of back-crossed $BALB/ct^{w32}$ mice were tested for their ZO₂ uptake in the presence of either lactate or pyruvate. The data show that the ZO₂ levels of the spermatozoa from $BALB/cT^+ | BALB/ct^{w32}$ and the spermatozoa from T^+/t^{w32} males were not significantly different in the presence of either substrate. Similarly, the spermatozoa from the back-crossed $BALB/cT^+ | BALB/cT^+$ litter mates did not differ significantly from either T^+/T^+ or T^+/T pooled spermatozoa in their utilization of these same substrates.

DISCUSSION

The present data show that recessive t^n -bearing spermatozoa are physiologically different from T^+ and T -bearing spermatozoa. Although Lyon, Glenister & Hawker (1972) have shown that the T locus is not necessary for successful

Table 1. A comparison of the transmission frequencies and NADH/NAD ratio of normal and mutant epididymal mouse spermatozoa

Genotype	No. of trials	Frequency of transmission	$\mu\text{g NADH}/10^8 \text{ sperm.}$	$\mu\text{g NAD}/10^8 \text{ sperm.}$	NADH/NAD ratio
T^+/T^+	8	0.50:0.50	17.9 $\pm$ 0.47	21.6 $\pm$ 0.18	0.80 $\pm$ 0.01
T^+/T	7	0.50:0.50	17.9 $\pm$ 0.26	21.8 $\pm$ 0.12	0.81 $\pm$ 0.01
T^+/t^6	7	0.10:0.90	15.1 $\pm$ 0.27	23.9 $\pm$ 0.52	0.61* $\pm$ 0.01
T^+/t^{w32}	7	0.22:0.78	16.3 $\pm$ 0.15	22.4 $\pm$ 0.11	0.68* $\pm$ 0.02

* Significant difference from normal controls at 5% level.

Table 2. A comparison of the oxygen uptake by pooled mouse spermatozoa in the presence of different substrates

Substrate (10 mM)	T^+/T^+	T^+/T	T^+/t^6	T^+/t^{w32}	$\frac{\text{BALB}/cT^+}{\text{BALB}/ct^{w32}}$	$\frac{\text{BALB}/cT^+}{\text{BALB}/cT^+}$
Glucose	11.94 $\pm$ 0.42†	11.15 $\pm$ 0.18	7.13 $\pm$ 0.42*	8.58 $\pm$ 0.27*	—	—
Fructose	12.46 $\pm$ 0.54	12.40 $\pm$ 0.21	14.46 $\pm$ 0.10*	14.20 $\pm$ 0.32*	—	—
Lactate	15.25 $\pm$ 0.44	15.73 $\pm$ 0.79	18.33 $\pm$ 0.65*	18.30 $\pm$ 0.53*	18.15 $\pm$ 0.46*	15.71 $\pm$ 0.21
Pyruvate	10.00 $\pm$ 0.28	9.00 $\pm$ 0.40	14.44 $\pm$ 0.64*	13.37 $\pm$ 0.55*	13.33 $\pm$ 0.61*	9.65 $\pm$ 0.35
Succinate	16.11 $\pm$ 2.70	17.86 $\pm$ 3.20	30.47 $\pm$ 1.30*	30.85 $\pm$ 2.10*	—	—

* Significant difference from control at 5% level.

† Values for ZO_2 are measured in terms of nmol $\text{O}_2/\text{hr}/10^6$ spermatozoa.

fertilization of ova, the results from the current studies show that the presence of the t -allele does increase the rate of sperm aerobic metabolism with most substrates. A comparison of pooled spermatozoa from $BALB/cT^+ / BALB/ct^{w32}$ males with spermatozoa from their $BALB/cT^+$ homozygous litter mates strongly indicates that the increased metabolism is a result of the presence of the meiotically driven allele. These males are 97% isogenic for all loci except those on Chromosome 17, which itself should be 97% isogenic for loci greater than ± 20 map units from the T -locus (Crow & Kimura, 1970).

This increased aerobic metabolism of t -spermatozoa could result in increased motility, capacitation and/or fertilization. Studies on the physiology of reproduction of mammals have shown that spermatozoa are passively transported, by peristaltic and antiperistaltic contraction waves, in both the uterus and the oviducal isthmus. There is some speculation, however, that spermatozoa must traverse the isthmo-ampullary junction to the site of fertilization by their own motility (Blandau, 1969). Time-lapse cinematography of the dispersal of a ribbon of spermatozoa from T^+/t^n and T^+/T^+ males does indicate a faster motility of t -bearing spermatozoa (Bryson, 1944). This increased motility, a function of increased metabolism, could therefore result in a disproportionate number of t -bearing spermatozoa being present at the site of fertilization.

It has recently been shown that the abilities to become capacitated and to fertilize are proportionally related to the levels of aerobic respiration and that these metabolic levels are, in turn, dependent upon the length of time spermatozoa remain in the female reproductive tract before fertilization (Hamner & Williams, 1963). It has also been shown that epididymal spermatozoa can be capacitated and can fertilize mouse ova *in vitro*, the percentage of capacitation and fertilization being substrate-dependent (Miyamoto & Chang, 1973). When incubated in a glucose-containing basic medium, only a small percentage of spermatozoa are capacitated and none fertilize ova. The addition of serum albumin to the basic medium increases both capacitation and fertilization, but not to the levels which are attained when either sodium lactate or sodium pyruvate is added to the albumin-fortified basic medium.

It is known that glucose is present in oviducal fluid (Bishop, 1957; Hamner & Williams, 1965; Holmdahl & Mastroianni, 1965), and although the present study shows that this substrate alone supports a higher rate of respiration in pooled spermatozoa from T^+/T^+ and T^+/T males than from pooled spermatozoa from T^+/t^n males, the fact that this substrate does not support capacitation and fertilization *in vitro* (Miyamoto & Chang, 1973) suggests that oviducal glucose is not normally used by spermatozoa as a metabolic substrate. Conversely, lactate and pyruvate, both known to be present in oviducal fluid (Bishop, 1957; Hamner & Williams, 1965; Holmdahl & Mastroianni, 1965; David, Brackett, Garcia & Mastroianni, 1969), and both known to promote capacitation and fertilization *in vitro* (Miyamoto & Chang, 1973), increase the rate of respiration of the mutant pooled spermatozoa significantly over that of pooled control spermatozoa. Fructose, the reducing sugar present in seminal fluid (Mann, 1946), causes a slight increase in O_2 uptake in sperm samples from T^+/t^n males above that of samples from either T^+/T^+ or T^+/T males. The greatest difference in ZO_2 between normal and t -bearing spermatozoa *in vitro*

is found when succinate is the sole energy source, but its presence in oviducal fluid has not yet been determined.

It has been reported that delayed matings will decrease the transmission frequency of *t*-bearing spermatozoa which are normally driven in spontaneous matings (Braden, 1958; Yanagisawa *et al.*, 1961; Erickson, 1973). For a higher rate of respiration to be effective in increasing capacitation and fertilization *in vivo*, spermatozoa must remain in the female reproductive tract for a period longer than 2 hr and less than 7 hr. Optimum capacity is reached after incubation for 6 hr (Hamner & Williams, 1963). In delayed matings, since the spermatozoa reside in the female for as little as 1 hr before fertilization (Braden, 1958), the increased respiration by the mutant-bearing spermatozoa over the T^+ -bearing spermatozoa may not be effective. Under these conditions, the *t*-bearing spermatozoa would not be 'driven' and ova would be fertilized by T^+ and *t*ⁿ spermatozoa equally. A longer sojourn of the spermatozoa in the female oviduct before fertilization would, on the other hand, enable the *t*-bearing spermatozoa to attain comparatively higher levels of respiration. The increased respiration would result in increased motility, capacitation and fertilizing ability, resulting in the meiotic drive of the *t*-bearing spermatozoa.

Although there have been several hypotheses advanced to explain meiotic drive (Zimmering, Sandler & Nicoletti, 1970), the present studies show that competition best fits the meiotic drive found in *t*-allele spermatozoa. Although all spermatozoa from T^+/t^n and T/t^n males are functional, the *t*-bearing spermatozoa are at a selective advantage because of their increased ability to utilize specific metabolic substrates aerobically. This competitive advantage may also be augmented by differences of membrane structure or antigenicity of the *t*-bearing spermatozoa (Bennett, Goldberg, Dunn & Boyse, 1972).

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