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THE *IN VITRO* EFFECT OF EXOGENOUS MELATONIN ON MOTILITY OF HUMAN SPERMATOZOA

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Abstract

Melatonin, a hormone secreted by the pineal gland is preesnt in the semen and melatonin binding sites have been demonstrated on the plasma membrane of sperm. These observations suggest a possible modulatory role of melatonin on sperm function. The aim of this study was to investigate the effects of melatonin on motility of human sperm *in vitro*. The concentration tested were 75, 150, 300, 450 and 900 pg mL^{-1} and the period of investigation was 120 min. The results show that, at all concentrations tested, melatonin suppressed motility of sperm. This antimotility effect was not dose-dependent but was time-dependent ; the greatest inhibition for a particular concentration being evident at the highest time point of incubation. Melatonin was not necrospermic but was only spermiostatic. Furthermore, it did not markedly alter the functional integrity of sperm of plasma membrane as revealed from the hypoosmotic swelling test. It is suggested that the sperm antimotility effect of melatonin may be due to its membrane stabilizing actions.

Key Words : Melatonin, human spermatozoa, sperm motility.

1. Introduction

Melatonin (N-acetyl - 5 - methoxy-tryptamine) is a hormone synthesised by the pineal gland which is released almost exclusively at night (Reiter, 1991). The pattern of the nocturnal blood melatonin rise, however, appears to be species specific (Reiter, 1991). Melatonin is highly lipophilic (Reiter., 1991) and this feature permits its rapid and easy access to many bodily fluids (Reiter, 1991) including semen (Reiter, 1991).

The presence of melatonin receptors have been reported in a variety of cells (Reiter, 1991), and last year, presence of melatonin-binding sites have been shown in human spermatozoa (van Vuuren, *et al.*, 1992). The role of melatonin in semen remains unestablished, but the presence of melatonin in semen (Reiter, 1991) and melatonin-binding sites on sperm plasmalemma

(van Vuuren, *et al.*, 1992) suggest that melatonin may act as physiological modulator of sperm motility. Since melatonin is known to exert an inhibitory effect on many reproductive processes (Reiter, 1991), it is likely that the modulatory role of melatonin may be a suppressive one. On the other hand, melatonin may induce a positive response on sperm motility. In complete contrast, sperm motility may be refractory to melatonin.

The present study was launched to investigate the effect of melatonin on motility of spermatizoa. This was carried out *in vitro* using unwashed human spermatozoa. The results obtained demonstrate that melatonin impairs motility of human sperm.

2. Materials and methods

Fresh semen specimens, obtained by masturbation with out lubricants from healthy adult volunteers (age 23-28 years), following at least 3-5 days of sexual abstinence were used. After being allowed to liquify (15-30 min) at room temperature (30-32°C) basic semen analysis were undertaken according to the protocol specified by the World Health Organization (1987). All ejaculates used ($n = 9$) had a volume of > 2.0 mL; sperm concentration $>40\times10_9$ spermatozoa mL^{-1}; motility of $> 50\%$; and normal morphology of $> 60\%$ These samples were diluted with isotonic saline (0.9% NaCl, w/v) so as to bring down the sperm concentration to 20×10^6 mL^{-1}, for the evaluation of the effects of melatonin on motility.

Melatonin (molecular weight 232g ; Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 100 uL of absolute ethanol and diluted with isotonic saline to obtain the desired concentrations. 500 uL of sperm suspensions were placed in Falcon tubes and an equal volume of either isotonic saline or test solution (final concentration 75, 150, 300, 450 and 900 pg mL^{-1}) was added, thoroughly mixed, and the time was recorded (Omin). These sperm suspensions were then incubated for 120 min at 30-32°C, and 10uL aliquots were transfered (at 0, + 10, +30, + 60 or + 120 min) onto separate clean glass slides at room temperature and covered immedately with a clean cover slip (22×22 mm).

The percentage motile spematozoa (WHO, 1987) was counted (approximately 100 cells) at each concentration and time point by a single observer under phase contrast optics (×400) using a squared grid (10×10 cm ; Fisons Scientific Equipment, Loughborough, UK).

The functional integrity of sperm plasma membrane was aessed using the hypoosmotic swelling test described by Jeyendran *et al.*, (1984) on melatonin-treated ($n = 6$; concentration 450 pg mlL^{-1}) and vehicle treated samples ($n = 6$) following 0 and +60 min of incubation at 30-31°C. All spermatozoa

showing characteristic swelling changes (curling of tails and/or shortning of tails) following incubation in the hypoosmotic medium were considered as having functionally normal plasma membranes. At least 100 spermatozoa were counted per preparation.

The viability of spermatozoa of melatonin-treated (n = 6 ; concentration 450 pg mL^{-1}) and vehicle-treated samples (n = 6) following Omin and +60min of incubation (at 30-31°C) was assessed using nigrosin-eosin stain technique (WHO, 1987). All spermatozoa showing any pink or red colouration in their head regions were classified as 'dead' and those appearing white or colourless as 'living'.

The data are presented as means $\pm$ EM. Data were statistically analysed by ANOVA and comparisons between control and test groups were made using Duncan's new multiple range test, except for hypoosmotic swelling and nigrosin-eosin tests where Mann-Whitney U-test was used. The significance level was fixed at $p < 0.05$

3. Results

Figure 1 depicts the effects of varing melatonin concentrations on human sperm motility with time (up to 120 min of incubation). The control-treatment had no significant ($p>0.05$) effect on sperm motility, except at 120 min, where the motility was significantly ($p<0.05$) impaired (by 18%). Such a deleterious effect with prolonged incubation is to be expected in view of the presence of naturally occuring sperm motility inhibitors in the seminal plasma (DeLamirande, *et al.*, 1984), and due to the proteolytic enzyme activity of the seminal fluid and or the exhaustion of sperm energy reserves (Huszar, *et al.*, 1990). Melatonin, on the otherhand, significantly ($p<0.05$) suppressed sperm motility (upto 19%) at all concentrations investigated. However, the antimotility effect of melatonin was not dose-dependent ($p>0.05$) and even at the highest concentration, it could not completely arrest sperm motility in any one of the samples. In contrast, the sperm motility inhibition capacity of melatonin was time-dependent ($p<0.05$); the greatest inhibition for a particular concentration being evident at the highest time point of incubation (see Figure 1). Another effect that was apparent with melatonin treatment was that with the reductions in percentage motility there was nearly always marked impairment in the progressive movement of sperm.

Table 1 summerises the results obtained with hypoomsotic swelling test and nigrosin-eosin stain technique. As shown, melatonin (450 pg mL^{-1} had no significant effect ($p>0.05$) either in the number of swollen spermatozoa (in the hypoosmotic medium) or 'living' spermatozoa (in nigrosin-eosin test).

4. Discussion

In this study, we assessed the influence of melatonin, a hormone secreted by the pineal gland, on human sperm motility (using fresh semen) by estimating the number of motile spermatozoa. Although, this technique is a subjective one, it is still widely used to evaluate the effects of various drugs (Seigal *et al.*, 1992 Ratnasooriya, *et al.*, 1992) and natural agents (Chogthammakun *et. al.*, 1986 ; Ratnasooriya, *et. al* 1993) on sperm motility. Our results clearly show that exogenous added melatonin, in physiological concentration, impairs motility of human spermatozoa *in vitro*. However, non of the concentrations of melatonin used in the present study completely blocked sperm motility. Since the initiation of this study Irez *et. al.*, (1992) have also demonstrated antimotility effects of melatonion on human sperm. However, they have investigated only three concentrations (150, 300 and 450 pg/mL^{-1}) in their study and also have not undertaken any additional experiments to ascertain the underlying mechanisms of the antimotility action (Irez *et al.*, 1992).

In this study, melatonin did not suppress the viability of spermatozoa as evident from nigrosin-eosin stain technique (WHO, 1987). Thus, melatonin is only spermiostatic and not necrospermic. The onset of the sperm antimotility effect of melatonin was extremely rapid; occurred as early as Omin of incubation. This observation indicated that the sperm plasmalemma is the most likely target for melatonin action. The presence of melatonin-binding sites on plasmalemma of human spermatozoa has been reported recently (van Vuuren *et al.*, 1992). Thus, it is most likely that melatonin binds to sperm in a receptor specific manner to initiate the observed antimotility effects. Furthermore, the fact that melatonin induced impairments in sperm motility at extremly low concentrations (in pg. level) lend support to a receptor mediated action rather than a non specific purtabation of the plasma membrane, a phenomenon, which can also inhibit sperm motility (Ratnasooriya, *et al.*, 1992). The antimotility effect of melatonin was not dose-related. This fact argues against a receptor mediated action. On the other hand, a lack of a dose-dependable response may result from homologous desensitisation of the receptor (if receptor mediation is presumed) (Briggs, *et al.*, 1981) or due to inverse agonism (Schoeifeld, 1989). A lack of a dose-dependent response may result from the presence of two sub populations of melatonin receptors having different affinities and producing opposite influences : such a situation is reported with respect to dopamine receptors in inducing penile erection in rats (Zarrindast *et al.*, 1992). A subsensitivity to higher concentrations of melatonin can also be resulted if melatonin is able to bind to other sperm receptors whose activation potentiate sperm motility : for instance, bovine placental lactogen can bind to prolactin and growth hormone receptors and activate them (Schaler *et al.*, 1992). Clarification on these possibilities, however, require additional studies.

Melatonin is a highly lipophilic hormone which is claimed to be a natural anticonvulcent agent (Sandyk *et al.*, 1992). Its anticonvulcent property is thought to be mediated through stabilizing effects on the electrical activity of the plasma membrane of the neurones of the central nervous system (Romijin, 1978). Drugs such as propranalol (Hong *et al.*, 1982a), quinidine, diltiazem (Hong *et al.*, 1984a), amitriptyline, imipramine (Hong *et al.*, 1984b) chloropromazine or phentoyn (Hong *et al.*, 1992b) which are known to possess membrane stabilizing effects inhibit motility of human sperm. Thus, it is reasonable to propose that membrane stabilizing effects of melatonin is the major factor, if not the only factor, responsible for the inhibition of sperm motility observed in this study. In the hypoosmotic swelling test (Jeyendarn *et al.*, 1984), melatonin did not reduce the number of swollen cells. Spermatozoa with a chemically and physically intact plasma membrane are claimed to show swelling under hypoosmotic conditions (Jeyendran *et al.*, 1992). Thus, melatonin - induced impairment in sperm motility cannot be attributed to any marked change in the functional integrity of the plasma membrane (Jeyendran *et al.*, 1992).

In this study, the severity of the antimotility action of melatonin increased with the time of incubation. This observation indicated that an enzymatic process may be involved in the melatonin-induced disruption in sperm motility. Since the induction of the antimotility action was almost instantaneous it is possible that melatonin may change the function of a membrane bound enzyme/s : several membrane bound enzymes are implicated with motility of sperm (Majumder *et. al.*, 1990). Alternatively, melatonin can penetrate into the sperm tail, [due to its high lipophilicity (Reiter, 1991)] and potentially interfere with protein phosphorylation [which is known to play a primary role in the second messenger regulatory mechanisms of flagellar axoneme-based movement (Tush 1989)], thereby impeding sperm movement : the demonstration of the presence of microtubule proteins with high specific affinity to melatonin (Winston *et al.*, 1974) and the ability of melatonin to markedly impair progresive movement of sperm (although not quantified) as evident in this study provide circumstantial evidence in support of this potential mechanism. In complete contrast, melatonin induced impairement in sperm motility may be due to other yet unknown physiological reasons.

In conclusion, the results of this study show that melatonin, a hormone secreted by the pineal gland, impairs motility of human sperm *in vitro*, at physiological concentrations. It seems, possible that melatonin may play a physiological role together with oxytocin (Ratnasooriya *et al.*, 1993) to keep sperm quiescent in the male genital tract until ejaculation. Melatonin is now known to be a multifunctional hormone (Ebadi *et al.*, 1993) and the proposed action suggested by us, on the basis of the results of this study, becomes yet another function assigned to this hormone. Because melatonin occurs in both male (Reiter, 1991) and female (Reiter, 1991) reproductive

systems and it can inhibit sperm motility and other reproductive parameters (Reiter, 1991) at extremely low concentrations, melatonin should be investigated, as a potential contraceptive, effective against both males and females.

Table 1 : Effects of Melatonin on the viability and hypoosmotic swelling of human sperm (Means ± SEM : n=6). The range of data values given in parantheses.

Incubation time	Control	Melatonin 450 pg mL^{-1}
%Number of viable spermatozoa		
0	68.67 ± 2.18 (60 — 75)	68.17 ± 0.70 (66 — 71)
60	69.84 ± 2.23 (60 — 75)	68.00 ± 1.91 (60 — 73)
%Number of swollen spermatozoa		
0	67.23 ± 1.98 (62 — 75)	64.00 ± 3.56 (47 — 71)
60	61.17 ± 2.63 (51 — 71)	57.50 ± 2.08 (50 — 65)

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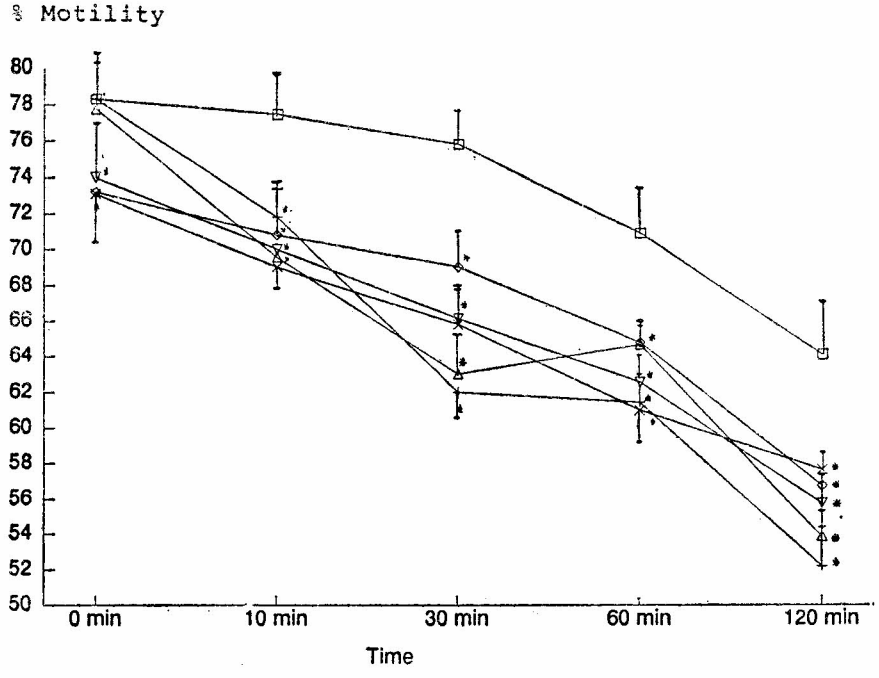

Figure 1. Inhibition of human spermatozoa by Melatonin

(Means ± SEM; n = 9) □ = Control ;
+ = 900 pg mL^{-1}; ◊ = 450 pg mL^{-1}; △ = 300 pg ml^{-1}
× = 150 pg mL^{-1}; ▽ = 75 pg mL^{-1}.

* As compared with venhicle control, p. < (0.05)

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