BIOLOGY OF REPRODUCTION 40, 197–200 (1989)

MINIREVIEW

Relaxin in the Male[1]

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ABSTRACT

Relaxin, a hormone usually associated with pregnancy, has been found in the semen plasma of many species. The prostate gland appears to be a source of relaxin. Relaxin stimulates sperm motility from suboptimal samples and increases sperm penetration into oocytes. Thus, relaxin may be an effective therapeutic agent in male infertility.

INTRODUCTION

Relaxin activity was first described by Hisaw (1926), but it was not until the 1970s that the structure of relaxin was determined. Relaxin is a peptide hormone with a molecular weight of 6000, with dissimilar A and B chains joined by two disulfide links. There is an additional disulfide link in the A chain. Relaxin is a structural homologue of insulin and the insulin-like growth factors (Schwabe and Harmon, 1978).

Human relaxin cDNA has been isolated, cloned, and sequenced. There are two nonallelic genes for human relaxin on the short arm of the ninth chromosome (Kemp and Niall, 1984). Relaxin has been clearly identified as a hormone of pregnant mammals. Many structural and functional changes of the reproductive tract during pregnancy have been ascribed to relaxin including cervical softening, uterine relaxation, and relaxation of the pelvic ligaments.

MALE RELAXIN

Occurrence of Relaxin in Males

Steinetz et al. (1959) identified relaxin bioactivity in rooster testis. Dubois and Dacheux (1978) described relaxin in boar testes. Using rabbit antirelaxin antiserum R6, an antibody raised in a rabbit against porcine relaxin but that recognizes relaxin from many mammalian species, Loumaye et al. (1980), described a relaxin-like substance in human seminal plasma. These results were confirmed by Essig et al. (1982b), who also demonstrated relaxin bioactivity in seminal plasma with the guinea pig pubic-symphysis palpation assay. These investigators hypothesized that the source of seminal relaxin is the prostate, since relaxin is found in semen from men after surgical vasectomy, which eliminates testicular and epididymal components from the ejaculate. Relaxin was found in the ejaculate of two men with congenital absence of the vas deferens and seminal vesicles. In these men, the ejaculate contained only components of the prostate and distal structures, yet the concentration of relaxin was higher than in most samples from normal men. These findings were confirmed by De Cooman et al. (1983), who also demonstrated higher concentrations of relaxin in the first portion of a human split ejaculate, lending support to an extra-gonadal source of relaxin. The first portion of a split ejaculate is usually rich in the prostatic contribution. By way of contrast, relaxin is not detectable in human male serum (O'Byrne et al., 1978). Juang et al. (1988) observed relaxin in the peripheral serum of boars, and reported that levels decrease after mating.

Using antirelaxin antibody R6, we have detected relaxin immunoactivity in the semen plasma of humans, baboons, monkeys, and boars, but not rats (unpublished). Cameron et al. (1982), using immunocytochemical technology, looked for relaxin activity in the prostate gland, seminal vesicles, testes, epididymis, and vas deferens of adult and prepubertal armadillos. Only prostatic epithelium from adult animals reacts with the relaxin antiserum. Dubois and Dacheux (1978) reported relaxin immunoactivity associated with interstitial and Sertoli cells of the testis, but these results could not be confirmed by Arakaki et al. (1980). Yamamoto et al. (1981) found very low

Accepted December 16, 1988.
Received December 12, 1988.
[1] Supported in part by NIH Grant HD 22338.

concentrations of relaxin immunoactivity in extracts of boar testes, casting doubt as to whether this structure is a source of relaxin. Kendall et al. (1983) reported detectable relaxin immunoactivity and bioactivity in adult human prostate extracts. Relaxin immunoactivity has been localized in the glandular epithelium of the prostate, seminal vesicle, and part of the vas deferens in men.

Relaxin has been partially purified from human seminal plasma by delipidation, high-performance liquid chromatography, ion-exchange chromatography, and gel filtration chromatography. Partially purifed human seminal relaxin is biologically active in inhibiting uterine motility in the rat uterine horn segment bioassay for relaxin. This material is also useful as an immunogen to produce antihuman seminal relaxin monoclonal antibodies (Weiss et al., 1986).

EFFECT OF RELAXIN ON SPERM

Motility

Relaxin affects sperm motility. An anti-porcine relaxin antiserum, but not normal rabbit serum, causes a rapid decline in the motility of washed human sperm. This effect is eliminated by preincubation of the antiserum with excess relaxin (Sarosi et al., 1983). A specific antirelaxin effect is supported by the observation that anti-insulin antiserum does not inhibit sperm motility in the same system (Lessing et al., 1984). Insulin is also present in seminal plasma. Sokol et al. (1988), using an antihuman relaxin antiserum, was able to rapidly inhibit the motility of human sperm, confirming the prior observations. Juang et al. (1987) inhibited the motility of boar sperm with antirelaxin antiserum. The immobilization of sperm can serve as a rapid screening test for antirelaxin antiserum. Relaxin antibodies may serve as adjuvants to increase the efficacy of barrier contraceptive methods.

Essig et al. (1982a), using washed human sperm resuspended in tris(hydroxymethyl)aminomethane-modified Ringers' saline solution or Bakers buffer, demonstrated that relaxin delays the loss of motility and grade of forward progression in ejaculated sperm that is seen after incubation over time. Normal semen samples were studied, and the positive effect of relaxin was fairly modest. Juang et al. (1987) demonstrated that relaxin can maintain porcine sperm motility.

To study further the effects of relaxin on human sperm motility, Lessing et al. (1986) examined both whole semen and washed spermatozoa from normal samples, normal samples aged for 5 h, and samples with initial low motility. The parameters selected were percent motility and grade of forward progression. Relaxin had no effect on the motility of whole normal semen specimen. The sperm-washing procedure decreased the motility of the normal samples. Motility in these samples was improved by the addition of either 10 or 100 ng/ml concentrations of porcine relaxin. Aging whole semen samples for 5 h at 37°C resulted in decreased motility. Relaxin in concentrations of 10 to 100 ml, the physiologic range, improved motility of these samples. Washing of the aged spermatozoa further decreased their motility. Relaxin did not improve the motility of these samples. When samples of initial low motility were studied, relaxin in the physiologic range significantly improved the motility of these samples. The washing procedure further depressed the motility of samples of initially low motility. Relaxin did not improve these samples' motility, suggesting that a level of damage can be reached from which recovery is no longer possible.

Since aging decreases the motility of normal semen samples, Lessing et al. (1986) hypothesized that relaxin in seminal plasma loses biological activity with aging. To test this hypothesis, normal semen samples were washed, aged for 5 h at 37°C and then treated with either their original seminal plasma, original seminal plasma with added relaxin, or fresh seminal plasma. Although the original seminal plasma had no significant effect on sperm motility, fresh seminal plasma or the original seminal plasma plus added relaxin significantly improved motility, thus supporting the authors' hypothesis.

In another study, Lessing et al. (1985) showed that cryopreservation of sperm in a 10% glycerol solution resulted in a decrease in post-thaw motility. This loss of motility was eliminated by pretreatment with caffeine, but not with relaxin. However, when relaxin was added to the sample after thawing, it resulted in improved motility of the sample. This also suggests that the biological activity of relaxin is adversely affected by freezing and thawing in seminal plasma.

To determine the effects of relaxin and prostaglandin E_2 (PGE_2) in combination, both components of seminal plasma reported to stimulate sperm motility, Colon et al. (1986a) added physiologic levels of relaxin, PGE_2, or the combination to either washed

or aged normal semen samples. Relaxin and PGE_2 individually improved the motility of washed spermatozoa. Relaxin, but not PGE_2, improved the motility of sperm in aged semen. This is consonant with the observation that relaxin activity decreases in semen plasma over time. In contrast to the observations with the individual substances, a combination of relaxin and PGE_2 had no effect on the motility of washed or aged spermatozoa. This suggests that either the two substances antagonize each other's action on sperm motility or the two agents affect sperm motility through the same mechanism. Since only optimal doses of both PGE_2 and relaxin affect sperm motility and doses that are too high or too low have no effect, it is possible that, in concert, these agents simply shift the curve to the right, thus being less effective than one agent alone.

In additional study, Park et al. (1988) confirmed the stimulatory effect of relaxin on sperm from samples of low motility or of aged sperm. Chan and Tang (1984), using washed normal samples aged with relaxin, were unable to show an effect on sperm motility. Both of these findings are consistent with the prior studies.

Brenner et al. (1987) studied relaxin content of 92 normal semen samples and 85 semen samples collected at random from infertile men. The mean relaxin concentration was roughly 45 ng/ml. The concentration of immunoreactive relaxin in the samples did not correlate with any of the parameters used for semen analysis: count, percent motility, grade of forward progression, volume, and morphology. This suggests that the biological activity of relaxin in ejaculated human semen is variable and not necessarily equivalent to the immunoactive relaxin. In contrast, relaxin concentration in boar seminal plasma correlates with sperm motility (Juang et al., 1988).

Porcine relaxin in physiologic concentrations significantly increases the penetration of human sperm into either human or bovine cervical mucus as compared to sperm treated with albumen or buffer (Brenner et al., 1984). Adding seminal plasma to freshly washed sperm equivalently increases the penetration into cervical mucus. Colon (unpublished results) has recently demonstrated that synthesized human relaxin increases the penetration of washed human sperm into bovine cervical mucus with a potency 10 to 100 times greater than that of porcine relaxin, and equivalent to that of whole semen. Since relaxin is a normal constituent of seminal plasma, human relaxin may be a safe and effective additive in the treatment of sperm used in the management of human infertility. Relaxin secretion in seminal plasma may represent a novel biological mechanism for hormone delivery. This exocrine male secretion is delivered to the female during coitus where it may facilitate sperm penetration through the female reproductive system.

Enhance Fertility of Oocytes

Pupula et al. (1986) demonstrated that porcine relaxin treatment enhanced fertilization of mouse oocytes by suboptimal concentration of spermatozoa in vitro. Park et al. (1987) also reported that incubation of mouse sperm with porcine relaxin increases fertilization rates of mouse oocytes in vitro. Park et al. (1988) showed that porcine relaxin enhances the penetration capability of human spermatozoa in the zona-free hamster egg penetration test. Relaxin was most effective in samples from men with low sperm counts and poor sperm motility. Only a minor augmentation of fertilization capability was noted in samples from normal fertile men. Chan and Tang (1984) studying samples from fertile men, were unable to find an effect of relaxin on a fertilizing capacity. This finding is consistent with the observations that relaxin is most effective in suboptimal sperm samples.

Colon et al. (1986b) hypothesized that the mechanism of action of relaxin on increasing sperm motility involves alterations in intracellular levels of cyclic adenosine 3′, 5′-monophosphate (cAMP). To test this hypothesis, cyclic AMP was measured in controls, and samples treated with relaxin, caffeine, and relaxin plus caffeine. Caffeine caused a significant increase in cyclic AMP levels. In contrast, relaxin did not increase the levels of intracellular cyclic AMP. The lack of an increase in the levels of cyclic AMP after treatment with relaxin plus caffeine above the levels after treatment with caffeine alone suggests that the mechanism of action of relaxin in improving sperm motility does not involve alterations in intracellular cAMP levels. This is consistent with the observation of Hildebrandt et al. (1985) that sperm cells do not contain stimulatory guanine nucleotide-binding regulatory components of adenylyl cyclase.

In summary, relaxin is present in seminal plasma in many species. Its source appears to be the prostate, but other sources in the male reproductive tract are also possible. Relaxin appears to affect human sperm motility and sperm-fertilizing capability in the

zona-free hamster egg-sperm penetration test, but only in suboptimal samples. Many questions remain to be answered. Is seminal relaxin a product of the same gene as ovarian relaxin, or is a different gene expressed in the male? What are the sources and roles of relaxin and what is its mechanism of action? Relaxin, however, appears to be a promising substance for the treatment of some causes of human male infertility.

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