Impaired Sperm Function After Spinal Cord Injury in the Rat Is Associated With Altered Cyclic Adenosine Monophosphate Signaling

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ABSTRACT: Our previous observations of changes in the expression of cAMP-dependent genes and the cAMP-responsive element modulator (CREM) in rat testicular cells after spinal cord injury (SCI) implied abnormal cAMP signaling as one of the mechanisms underlying the effects of SCI on spermatogenesis. It was postulated that such effects might contribute to abnormal sperm function after SCI. In this study, we examined this possibility. In spinal cord–contused (SCC) and –transected (SCX) rats, impaired sperm motility was accompanied by an increase in sperm cAMP content. Treatment of SCX rats with exogenous testosterone or follicle-stimulating hormone resulted in a further decrease in sperm motility, whereas sperm cAMP either increased or remained unchanged. These effects differed from those in sham control rats that received identical treatments. Results of these experiments also demonstrated that impaired sperm motility in SCC and SCX rats was accompanied by decreases in sperm viability and mitochondrial potential, thus suggesting a possible link between these changes. We concluded that impaired sperm motility after SCI was associated with decreases in sperm viability and mitochondrial potential. These effects occurred in the face of elevated sperm cAMP content and changes in its regulation, suggesting that altered cAMP signaling events might contribute to impairment of sperm motility and perhaps other sperm functions after SCI.

Key words: cAMP, SYBR-14, JC-1.
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Male infertility resulting from spinal cord injury (SCI) is associated with abnormal semen parameters. Although decreases in progressive sperm motility and sperm with normal morphology were common, findings regarding the effect of SCI on sperm production varied among studies (Hirsch et al., 1991; Linsenmeyer and Perkash, 1991; Brackett et al., 1994). These results suggest that multiple steps in spermatogenesis, sperm maturation, or both might be affected by cord injury. Our earlier studies demonstrated that both endocrine- and neural-related mechanisms were involved in the effects of SCI on spermatogenesis (Huang et al., 1995, 1998; Chow et al., 2000). Recent observations, including changes in expression and hormone regulation of cAMP-dependent genes and expression and cellular distribution of the cyclic AMP responsive element (CRE) modulator (CREM) in spermatogenic cells (Huang et al., 2003a,b, 2004a,b), strongly suggest that alteration in cAMP-dependent functions of spermatogenic cells might contribute to impaired spermatogenesis after SCI.

Postmeiotic differentiation of spermatids is stringently regulated by germ cell–specific genes leading to the development of specialized organelles essential for sperm function and species-specific sperm morphology (Hecht, 1990; Steger, 1999). The promoter of many of these genes, including lactate dehydrogenase C, transition protein 1, and protamine, contained CRE (Kistler et al., 1994; Ha et al., 1997; Bonny et al., 1998), suggesting that their expression and cellular effects might be modulated by cAMP. In addition, spermatids express transcripts for cAMP-dependent protein kinase subunits (Oyen et al., 1990) and protein kinase A anchor proteins that have localized in the sperm tail (Miki and Eddy, 1998; Moss et al., 1999). These findings and the vital importance of cAMP–protein kinase signaling events in various sperm functions (Vijayaraghavan and Hoskins, 1986; Galantino-Homer et al., 1997; Visconti and Kopf, 1998) led us to postulate that impaired cAMP signaling events and their cellular effects might also contribute to abnormal sperm function after SCI. Furthermore, persistently lower sperm motility in cord-transected SCI rats after a prolonged recovery period was associated with reduced sperm viabil-
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ity and mitochondrial potential (Huang et al., 2004a). In this study, we compared the relationship between sperm motility and sperm cAMP content, viability, and mitochondrial potential in rat subjected to various potentially reversible cord injuries by contusion and subjected to irreversible cord injury by transection. We further examined the effects of exogenous testosterone and follicle-stimulating hormone (FSH) on these parameters in cord-transsected rats.

Materials and Methods

Animals

Mature Sprague-Dawley rats (300–350 g, Taconic Farm, Taconic, NY) were caged individually in an air-conditioned, light-controlled animal room for 2 weeks before the experiment. They were fed Purina rat chow and water ad libitum.

Spinal Cord Injury

For surgically induced cord injury, the rats were anesthetized with sodium pentobarbital (45 mg/kg), and the spinal cord was exposed at the T9–T10 vertebra level by laminectomy. Spinal cords were variously contused (SCC rats) with a rod dropped from different heights (12.5, 25, 50, or 75 mm) with an NYU IMPACTOR (Kwo et al., 1989), or they were surgically transected (SCX rats) as previously described (Linsenmeyer et al., 1994). The muscle layer was sutured and the wound closed with surgical clips. Sham control rats received a sham operation without laminectomy. The surgical procedures for cord transection and contusion were reviewed annually and approved by the Institutional Animal Care and Use Committees at both the East Orange Veterans Affairs Medical Center (New Jersey) and University of Medicine & Dentistry, New Jersey Medical School. Postoperative care procedures for SCC and SCX rats (Linsenmeyer et al., 1994) were followed to maintain general health of the animal.

Cohorts of SCC rats (n = 6–8 per group) and sham control rats (n = 7) were killed 8 weeks postinjury, a time at which impaired sperm motility was inversely correlated with the extent of injury. A follow-up experiment was subsequently performed 4 weeks postinjury, a time at which impaired sperm motility was not related to the extent of cord injury (Huang et al., 2003b).

Administration of Exogenous Hormones

The SCX rats (n = 5–7 per group) and sham control rats (n = 5 per group) were given subcutaneous (SC) implants of testosterone-filled silastic capsules (TC, 1–10 cm) (Huang et al., 2004b) in the flank region immediately after surgery. Animals were sacrificed 8 weeks later, a time at which the effects of exogenous testosterone hormones were previously demonstrated (Huang et al., 1999, 2004b). Because short-term treatment of SCX rats with exogenous FSH enhanced spermatogenic regression (Huang et al., 1999), a group of SCX (n = 7) and sham control rats (n = 5) were given daily SC injections of 0.5 IU porcine FSH (Sigma Chemicals, St Louis, Mo) for 2 weeks before sacrifice. Because testosterone and FSH were administered to maintain spermatogenesis in cord-transected SCI rats (Huang et al., 1999) but spermatogenesis was maintained in all SCC rats (Huang et al., 2003b), the effects of exogenous hormones were only examined in SCX rats.

Collection of Sperm

The epididymis was dissected immediately from the testis after sacrifice and bisected into the caput and caudal portions at the middle of the organ for collection of sperm in different maturation stages. One caudal epididymal was immediately immersed in 4–5 mL of 37°C Kreb Ringers solution supplemented with sodium pyruvate (1 mM), d-glucose (5.57 mM), sodium bicarbonate (10 mM), N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES, 25 mM), and bovine serum albumin (BSA, 2%). The distal end of the caudal epididymis was punctured at 20–30 locations with a 19-gauge needle, and spermatozoa were flushed out with a gentle stream of buffer and transferred to a new petri dish. The sperm suspensions from each rat were kept at 37°C in a CO2 incubator, and motility was examined within 10–15 minutes. The remaining sperm specimens were maintained for an additional 45–60 minutes until all rats were sacrificed for subsequent measurement of viability and mitochondrial potential. Routinely, 4–6 rats were sacrificed each day, and preliminary experiments revealed that under the conditions employed, sperm viability and mitochondrial potential of sham control rats remained relatively unchanged during the first 90–120 minutes. Coefficient of variation of these parameters among sham control rats killed at different dates was within 10%.

Both caput epididymides and the second caudal epididymis from each rat were immersed in 4–5 mL of ice-cold phosphate-buffered saline (PBS). The tissues were then sliced with a scalpel 8–10 times in 5 mL of buffer in a petri dish to release spermatozoa. Sperm suspensions were transferred to 15-mL tubes and vortexed, and tissue fragments were allowed to settle for 3–5 minutes. Thereafter, sperm suspensions were transferred to new tubes, and spermatozoa were collected by centrifugation (200 x g for 5 minutes), washed with PBS, and counted. Aliquots of 1 x 106 spermatozoa were pelleted in 1.5-mL microfuge tubes, frozen on dry ice, and stored at −80°C for subsequent measurement of cAMP and protein phosphorylation.

Sperm Motility

A drop (50 µL) of caudal sperm suspension from each rat was placed on a prewarmed hemocytometer and allowed to settle for approximately 1 minute. Spermatid in 10–20 microscopic fields were examined and videotaped with a Nikon research microscope equipped with an image capturing system. Sperm motility was evaluated at a later time. A sperm was considered "motile" when its tail oscillated and did not remain at the same location during the 5–10-second taping period.

Sperm Viability and Mitochondrial Potential

A Becton Dickinson FACScan flow cytometer (BD Biosciences, San Jose, Calif) was used to measure sperm uptake of SYBR-14 and JC-1 fluorescent dye (Molecular Probes, Eugene, Ore) specific for viability and mitochondrial potential (Graham et al., 1990; Gravance et al., 2001). The electronics of the instrument
were optimized each day before the sacrifice of a cohort of 4–6 rats by the following procedure. Aliquots of 250 μL of caudal sperm suspension of a sham control rat were frozen and thawed 3 times in a mixture of dry ice and isopropanol and were gated on FL3 (propidium iodide) to identify the majority of the sperm cells. They were back-gated on forward vs side scatter for acquisition of the remaining gated samples. Acquisitions and analyses of at least 50,000 gated events were performed with CellQuest (Becton Dickinson) and FlowJo (TreeStar Inc, Ashland, Ore) software.

For measurement of viability and mitochondrial potential, 0.5 mL aliquots of caudal sperm suspension from each rat were placed in polystyrene Falcon tubes (12 × 75 mm) in triplicate and stained with 5 μL of SYBR-14 diluted in SYBR buffer (0.15 M saline, 10% bovine serum albumin [BSA], and 10 mM HEPES), 5 μL of SYBR-14 plus 5 μL of propidium iodide dissolved in dimethyl sulfoxide (DMSO), or 2 μL of JC-1 dissolved in DMSO, for 15 minutes at 37°C. Acquisitions and analyses of at least 50,000 gated events were performed as described above, and statistical analyses of fluorescence data were performed with InStat3 software (GraphPad Software, San Diego, Calif).

**Measurement of cAMP**

Sperm cAMP was extracted and measured according to the procedures described by Wu et al (1995). Briefly, precounted sperm pellets (1 × 10⁴) were resuspended in 0.2 mL of PBS and mixed with 0.8 mL of ice-cold 5% trichloroacetic acid. After centrifugation at 200 × g for 5 minutes, the supernatants were acidified by adding 125 μL of 1 N hydrochloric acid in 10-mL capped glass centrifuge tubes and vortexed. The samples were extracted 3 times with 2 mL of ethyl ether, and the ether fraction was removed after centrifugation at 800 × g for 5 minutes. The aqueous phase after the last ether extraction was frozen and lyophilized overnight with a SpeedVac Concentrator (Medical Resources, San Antonio, Tex). Pilot experiments revealed that 85%–90% of βH-cAMP added to sperm suspensions or aliquots of cAMP standard solution were recovered by this procedure consistently. The lyophilized samples were redissolved in 150 μL of 0.2 M sodium acetate (pH 4.7). Duplicates of 50-μL samples were mixed with 100-μL reaction mixtures containing βH-cAMP, histone/water (1/1), vol/vol, and 50 μL of protein kinase A (142 ng/50 μL) and then incubated for 1.5 hours on ice. Thereafter, 100 μL of hydroxyapatite (12.5% in 10 mM potassium phosphate buffer [KPB]) was added to each tube and incubated on ice for 10 minutes. Each tube was then washed 3 times with 2 mL of KPB and centrifuged, and the supernatant was discarded. Subsequently, the pellet was dissolved in 100 μL of 3 N hydrochloric acid by vortex, and radioactivity was counted in 5 mL of scintillation fluid with a Beckman LS 5000TD scintillation counter (Beckman Coulter, Inc, Fullerton, Calif). The concentration of cAMP in each sample was then calculated against a standard curve generated for each assay.

**Sperm Protein Phosphorylation**

Sperm pellets were dissolved in 50 μL of lysis buffer (1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS] in PBS) and boiled at 95°C for 5 minutes. After centrifugation at 20,000 × g for 5 minutes, 1 μL of the supernatant of each sample was diluted in 100 μL of water for protein concentration determination. The protein extracts were diluted with lysis buffer to a final concentration of 40 μg protein per 10 μL, mixed and an equal volume of 2× loading buffer (62.5 mM Tris, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, pH 6.8), and boiled at 95°C for 5 minutes. Aliquots containing 20 μg of protein were then electrophoresed on a standard 10% SDS polyacrylamide gel. Prestained molecular mass markers (Bio-Rad Laboratories, Hercules, Calif) were included in each gel. Subsequently, proteins were electroblotted onto PVDF membrane (Bio-Rad Laboratories, Hercules, Calif) and immunostained with anti-phosphotyrosine monoclonal antibody (1:1000, Santa Cruz Biotech, Santa Cruz, Calif) according to standard procedures. The phosphorylated protein bands were visualized with Chemiluminescence Reagent (Perkin Elmer Life Sciences, Boston, Mass).

**Statistics**

All data were evaluated for normal distribution, and analysis of variance was employed to detect effects. When the treatment effects were significant (P < .05), planned a priori comparisons were made with Dunn’s tests to determine the statistical significance of differences among treatment groups.

**Results**

**Effects of Cord Contusion on Sperm Function**

Eight weeks after cord contusion, caudal sperm motility of SCC rats was significantly decreased (P < .05; Figure 1A) and inversely correlated with height of the weight drop, consistent with previous findings (Huang et al, 2003b). Unexpectedly, a significant increase in cAMP content was detected in the caudal sperm of SCC rats injured by a weight dropped from heights of 25 to 75 mm (P < .05; Figure 1B). Cyclic AMP levels of caput epididymal sperm of SCC rats, however, remained comparable to that of sham control rats. Because reduced sperm viability and mitochondrial potential were attributed to lowered sperm motility in SCX rats (Huang et al, 2004a), we also examined sperm uptake of SYBR-14 and JC-1, fluorescent dyes specific for viability and mitochondrial potential, in SCC rats that received weight dropped from 25 and 75 mm. As shown in Figure 1C, sperm uptake of SYBR-14 and JC-1 was significantly reduced in both groups of SCC rats (P < .01).

A follow-up experiment was undertaken to determine the relationship between sperm cAMP and motility at an earlier time (4 weeks postinjury). Because sperm motility was not related to the extent of cord injury at this time (Huang et al, 2003b), only rats injured by a weight dropped from 25 and 75 mm were examined. Figure 2A shows that motility of caudal sperm was significantly reduced in both groups of SCC rats (P < .01). In these rats, cAMP content in caput sperm increased significantly (P < .05; Figure 2B). Cyclic cAMP content in caudal sperm
Effects of Exogenous Hormones on Sperm Functions

To determine whether cAMP-related events, viability, and mitochondrial potential were also involved in hormone effects on sperm motility, these parameters were examined in spermatozoa of SCX rats that received exogenous testosterone or FSH treatments. Eight weeks after injury, sperm motility in untreated SCX rats was significantly lower than in untreated shams (P < .05). Implantation of 1-10-cm TC resulted in slight but dose-dependent decreases in sperm motility in sham control rats receiving 5- or 10-cm TC implants (Figure 3A; P < .05). Similarly, TC implantation also resulted in further decreases in sperm motility in SCX rats, especially in those that received a 2-cm TC implant (P < .01). Sperm motility in SCX rats receiving 5- or 10-cm TC implants rebounded but remained statistically lower in SCX rats receiving 5-cm TC implants when compared with untreated SCX rats and their sham control counterparts (P < .05,
Figure 3. The effect of exogenous testosterone and follicle-stimulating hormone (FSH) on sperm function in spinal cord–transected (SCX) rats 8 weeks postinjury. (A) Sperm motility was reduced in sham control rats and SCX rats receiving testosterone-filled silastic capsule (TC) implants or FSH injections, but the extent of decrease differed significantly between the 2 groups. (B) Sperm cAMP content was higher in SCX rats and was further increased in those SCX rats receiving 1- or 2-cm TC implants but was not changed in those receiving 5- or 10-cm TC implants. In contrast, sperm cAMP was significantly increased in sham control rats receiving 5- or 10-cm TC implants. FSH also stimulated sperm cAMP in sham control rats but did not affect sperm cAMP significantly in SCX rats. Results are expressed as mean ± SEM, n = 5–7 rats per group. a, P < .05; aa, P < .01 vs untreated sham control; b, P < .05; bb, P < .01 vs untreated spinal cord injury.

P < .01, respectively). Daily injection of FSH for 2 weeks also reduced sperm motility in sham control rats (P < .05) and eliminated sperm motility in SCX rats (P < .01).

As in SCC rats, cAMP level in the caudal sperm of SCX rats increased despite a statistical significance (Figure 3B). Administration of exogenous testosterone resulted in an increase in sperm cAMP level in sham control rats that received 5- or 10-cm TC implants (P < .05). Sperm cAMP level also increased in SCX rats receiving 1- or 2-cm TC implants to levels that were significantly higher than in untreated sham control rats (P < .05 and .01, respectively). In contrast, sperm cAMP level in SCX rats was not affected by 5- or 10-cm TC implants. FSH injections also elevated sperm cAMP slightly in sham control rats (P < .05) but suppressed that in SCX rats by 30% despite a lack of statistical significance.

Flow cytometric analysis revealed a significant decrease in uptake of SYBR-14 and JC-1 by spermatozoa of SCX rats (P < .01; Figure 4). Implantation of 10-cm TC implants did not affect SYBR-14 and JC-1 uptake by spermatozoa of sham control rats but did restore uptake in SCX rats (P < .01).

**Sperm Protein Phosphorylation**

Western blotting revealed at least 6 phosphorylated protein bands in the sperm of sham control rats, with the most prominent band at a molecular mass of between 93 and 115 kd (Figure 5A). Spinal cord transection resulted in a greater than 20% decrease in this major band (P < .05; Figure 5B) and decreases in most other protein bands. Administration of exogenous testosterone resulted in increases in most phosphorylated protein bands in sham control and SCX rats (Figure 5C and D). The increase in the major protein band in testosterone-treated SCX rats, when normalized against untreated SCX rats, was comparable to that in sham control rats (Figure 5E). FSH treatment did not affect sperm protein phosphorylation significantly.

**Discussion**

Impairment of sperm function is well documented as a consequence of SCI. However, little is known about the underlying causes for such effects. Several SCI-related health problems, including malnutrition and stress, were known to affect sperm function, including motility. However, because body weights of SCC and SCX rats were within the range of normal mature male rats at the time of sacrifice (data not shown), the abnormal sperm motility seen in SCC and SCX rats cannot be ascribed to malnutrition. Undoubtedly SCC and SCX rats were under constant stress because of a lack of normal mobility. However, daily observation of stress-related indicators, such as alertness, fur condition, and cleanliness of the eye lid, indicated that the stress these animals were experiencing was minimal. These observations suggest that stress was not likely the cause for decreased sperm motility in SCC and SCX rats. Differences in sperm motility in SCX rats receiving various doses of exogenous testosterone support this notion.

Previously, we found that sperm motility in SCC rats that suffered less injury (ie, rats that received weight dropped once from a 12.5- or 25-mm height) recovered during the chronic phase of the injury and rats that suffered more severe injuries continued to deteriorate over time (Huang et al, 2003b). The latter is consistent with what occurred in SCX rats in this study (Huang et al, 2004a,b). We postulate that factors intrinsic to the sperm might dictate recovery or continuous deterioration of sperm motility (and perhaps other sperm function) over time in rats that suffered different extents of cord injury. Because of the importance of cAMP in various sperm functions, we sought to determine whether impaired
sperm motility following SCI was due to a decrease in sperm cAMP. In chronic SCX rats, decreases in sperm motility were associated with reduced viable sperm and lower mitochondrial potential (Huang et al., 2004a). We also examined sperm viability and mitochondrial potential in SCC and SCX rats under various experimental conditions.

A negative correlation between sperm motility and the extent of cord injury in SCC rats 8 weeks postinjury was consistent with previous results (Huang et al., 2003b). Because sperm motility was suppressed to a greater extent 4 weeks after SCC rats were injured by a weight dropped from both 25 and 75 mm, the higher sperm motility seen at 8 weeks in SCC rats of the 25-mm group was consistent with its recovery. Concomitant improvement of sperm viability and mitochondrial potential in SCC rats of the 25-mm group at this time suggests that they were involved in the effects of cord injury on sperm motility. These effects could be attributed to faulty development of the sperm from abnormal spermiogenesis or abnormal sperm maturation because of impaired epididymal function (Ricker et al., 1996; Kempinas et al., 1998).

The dose-related decreases in sperm motility in sham control rats and SCX rats given exogenous testosterone cannot be ascribed to the status of epididymal functions; they should have been better preserved in rats with the 5- or 10-cm TC implants because they had higher serum and testicular testosterone levels when compared with those that received the 1- or 2-cm TC implants (Huang et al., 2004b). These effects were probably unrelated to sperm viability or mitochondrial potential because 10-cm TC implants did not affect sperm viability and mitocho-
Figure 5. Effect of spinal cord transaction (SCX) and exogenous hormones on sperm protein phosphorylation. (A) A representative Western blot showing lower intensities of phosphorylated protein bands in spermatozoa of SCX rats. (B) Quantitative comparison of intensity of the major phosphorylated sperm protein between sham control and SCX rats. Results are expressed as mean ± SEM of 4 rats. a, P < .05 vs sham control. (C, D) Representative Western blots of sham control and SCX rats, respectively, receiving various hormone treatments. Each blot contained samples randomly selected from each experimental group. (E) Intensity of the major phosphorylated protein band was scanned and normalized against that of the untreated control in each blot. Results are expressed as mean ± SEM of 4 blots.

Drial function in sham control rats but significantly improved both in SCX rats.

A higher cAMP content in the sperm of SCC and SCX rats was unexpected in the face of lowered sperm motility. Such increases were probably unrelated to hormone status in these rats because the pituitary-testis hormone axis has recovered during the chronic phase of the injury (Huang et al., 1995, 2004b). This effect was more pronounced in the sperm of SCX rats receiving a 2-cm TC implant that had severely impaired motility and was also observed in sham control rats receiving 5- or 10-cm TC implants that had reduced sperm motility. Of interest, sperm cAMP content in SCX rats was not affected by 5- and 10-cm TC implants, and motility of these sperm was better maintained when compared with the rats receiving a 2-cm TC implant. Changes in the response of sperm cAMP content to exogenous testosterone in SCX rats, nevertheless, suggest that cAMP production was perturbed after cord injury. These results were incompatible with the dogma emphasizing a link between sperm cAMP production and sperm motility, suggesting that changes in signaling events downstream of cAMP production might account for impairment of sperm motility after cord injury. This notion is corroborated by differences in sperm motility between sham control rats and SCX rats that received identical FSH treatment and had comparable sperm cAMP content. The effects of FSH were most likely mediated by testosterone because identical FSH treatments enhanced testicular accumulation of testosterone (Huang et al., 1991) that could affect spermiogenesis or epididymal functions.

The steady state cellular level of cAMP is regulated by multiple adenylate cyclases (ACs; Collins et al., 1991; Taussig and Gilman, 1995) and phosphodiesterases (PDEs; Beavo 1995; Conti et al., 1995), many of which have been detected in testicular cells (Kopf and Vacquier, 1984; Rojas et al., 1993; Salanova et al., 1999). cAMP signaling in the sperm is modulated by the G-protein-mediated membrane-bond ACIII and a soluble AC (sAC), and the function of these ACs was regulated by capitation agents such as Ca2+-calmodulin and bicarbonate (Kopf and Vacquier, 1984; Okamura et al., 1991; Rojas et al., 1993, Jaiswal and Conti, 2003). In addition, presence of multiple PDEs in human sperm might provide compartmentalized cAMP pools for specific functions (Fisch et al., 1998).

In the sperm, cAMP activates specific protein kinases involved in various sperm functions. Phosphorylation of flagellar proteins initiates sperm motility during epididymal maturation (Hoskins et al., 1974; Vijayaraghavan and Hoskins, 1986; Bracho et al., 1998) and hyperactivated motility during capacitation (Visconti and Kopf, 1998; Mahony and Gwathmey, 1999; Adeoye-Osiguvara and Fraser, 2002). The cAMP/protein kinase A signaling events are also involved in regulating the redistribution of phospholipid and cholesterol within the plasma membrane preceding the acrosome reaction (Gadella and Harrison, 2000), reiterating the importance of cAMP signaling events in various sperm functions leading to fertilization.

An overall decrease in sperm protein phosphorylation in SCX rats while their sperm cAMP contents were elevated suggests a dysfunctional cAMP–protein kinase cascade that might contribute to impaired sperm motility and perhaps other sperm functions. However, the extent of
changes in sperm protein phosphorylation in hormone-treated SCX rats were comparable to that in their sham control counterparts, suggesting that the majority of sperm phosphorylated proteins were able to respond to exogenous hormones normally after SCX. Recent studies demonstrated that bicarbonate stimulation of sperm motility was mediated by the sperm sAC, and sperm lacking sAC exhibited severe motility defects and were infertile (Esposito et al., 2004; Luconi et al., 2005). Understanding the effect of SCI on sperm ACs, their signaling, and downstream cellular effects could hold the key to unraveling the mechanisms responsible for abnormal sperm function after cord injury.

Decreases in sperm motility in the rat after spinal cord injury were associated with parallel decreases in sperm viability and mitochondrial potential. An elevated sperm cAMP level and an overall decrease in sperm protein phosphorylation in SCC and SCX rats suggest that sperm cAMP–protein kinase signaling events were perturbed after cord injury. Although exogenous testosterone maintained sperm viability and mitochondrial potential in SCX rats, lack of a concomitant effect in sperm motility suggests that multiple mechanisms were involved in the effects of SCI on sperm function.

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References


