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 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 may contribute to abnormal sperm functions after SCI. The current study examined this possibility. In spinal cord contused (SCC) and transected (SCX) rats impaired sperm motility was accompanied by an increase in sperm cAMP contents. Treatment of SCX rats with exogenous testosterone or FSH resulted in further decrease in sperm motility while sperm cAMP was either increased or remained unchanged. These effects differed from that in sham control rats 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 cause-effect relationship between these changes. Conclusion: Decreases in sperm viability and mitochondrial potential contributed to impaired sperm motility after SCI. These effects occurred in the face of elevated sperm cAMP content and changes in its regulation, suggesting that altered cAMP signaling events also contributed to impairment of sperm motility, and perhaps other sperm functions after SCI.

Key words: spinal cord injury, sperm, cAMP, SYBR-14, JC-1

Introduction

Male infertility resulting from spinal cord injury (SCI) is associated with abnormal semen parameters including decreases in sperm count and progressive motility, and fewer sperm with normal morphology (Hirsch et al., 1991; Linsenmeyer and Perkash, 1991; Brackett et al., 1994). These results suggest that multiple steps of spermatogenesis and/or sperm maturation 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 the expression and hormone regulation of cAMP-dependent genes, and expression and cellular distribution of cyclic AMP responsive element (CRE) modulator (CREM) in spermatogenic cells (Huang et al., 2003a,b; Huang et al., 2004a,b), strongly suggest that alteration in cAMP-dependent functions of spermatogenic cells might contribute to impaired spermatogenesis after SCI.

Post meiotic 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 (Bonny et al., 1998; Kistler et al., 1994; Ha et al., 1997), suggesting that their expression and cellular effects might be modulated by cAMP. In addition, spermatids express transcripts for cAMP-dependent protein kinase sub-units (Oyen et al., 1990) and protein kinase A anchor proteins which have been localized to sperm tail (Miki and Eddy, 1998; Moss et al., 1999). These findings and 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 may also contribute to abnormal sperm functions after SCI. Furthermore, persistently lower sperm motility in cord-transected SCI rats after prolonged recovery period was associated with reduced sperm viability and mitochondrial potential (Huang et al., 2004b). In the current study, we compared the relationship between sperm motility and sperm cAMP content, viability and mitochondrial potential in the rat subjected to various extents and potentially reversible cord injury by contusion, and those that subjected to irreversible cord injury by transection. We further examined the effects of exogenous testosterone and FSH on these parameters in cord-transected rats.

Materials and Methods

Animals

Mature Sprague Dawley rats (300-350 gm, Taconic Farm, Taconic, NY) were caged individually in an air-conditioned, light-controlled animal room for two weeks prior to 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 level of T9-T10 vertebra by laminectomy. The spinal cord was contused to various extents by a rod dropped from different heights (12.5, 25, 50 or 75 mm, SCC rat) using an NYU IMPACTOR (Kwo et al., 1989), or surgically transected (SCX rat) as previously described (Linsenmeyer et al., 1994). The muscle layer was sutured and the wound closed with surgical clips. Sham control rats received sham operation without laminectomy. The surgical procedures for

cord transcetion and contusion were reviewed annually and approved by the Institutional Animal Care and Use Committees at both the East Orange VA Medical Center and UMD-New Jersey Medical School. The procedures of post-operative cares for SCC and SCI rats (Linsenmeyer et al., 1994) were followed to maintain general health of the animal.

Effects of the extent of cord injury

Cohorts of SCC rats and sham control rats were killed 8 weeks post injury, a time that impaired sperm motility was inversely correlated with the extent of injury. A follow-up experiment was subsequently performed 4 weeks post injury, a time that impaired sperm motility was not related to the extent of cord injury (Huang et al., 2003b).

The effects of exogenous hormones

The SCX rats and sham control rats were given subcutaneous (s.c.) implants of testosterone-filled silastic capsules (TC, 1-10 cm) (Huang et al., 2004a) in the flank region immediately after the surgery. Animals were sacrificed 8 weeks later, a time that the effects of exogenous testosterone hormones were demonstrated (Huang et al., 1999, 2004b). Because short term treatment of SCX rats with exogenous FSH enhanced spermatogenic regression (Huang et al., 1999), a small group of SCX and sham control rats were given daily s.c. injection of 0.5 I.U. porcine FSH (Sigma Chemicals, St Louis, MO) for 2 weeks prior to sacrifice. Since testosterone and FSH were administered for the purpose 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 of different maturation states. One caudal epididymidis was immediately immersed in 4-5 ml 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-ethanessulfonic acid] (HEPES, 25 mM) and bovine serum albumin (BSA, 2%). The distal end of the caudal epididymidis was punctured at 20-30 locations with a 19 G needle, and spermatozoa were flushed out with a gentle stream of the buffer and transferred to a new Petri dish. The sperm suspensions from each rat were kept at 37° C in a CO_2 incubator, and motility was examined within 10-15 minutes. The remaining sperm specimens were maintained for 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. Co-efficiency of variation of these parameters among sham control rats killed at different dates was within 10%.

Both caput epididymides and the second caudal epididymidis from each rat were immersed in 4-5 ml ice cold phosphate buffered saline (PBS). The tissues were then sliced with a scalpel 8-10 times in 5 ml 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 (1000 rpm x 5 minutes), washed with PBS and counted. Aliquots of $1x10^7$ 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 daudal sperm suspension from each rat was placed on a pre-warmed hemocytometer and allowed to settle for approximately 1 minute. Spermatozoa in 10 - 20 microscopic fields were examined and video-taped using a Nikon research microscope equipped with image capturing system. Sperm motility was evaluated at later times. A sperm was considered "motile" when its tail oscillated and did not remain at the same location during the 5-10 second taping time.

Sperm viability and mitochondrial potential

A Becton-Dickinson FACScan flow cytometer was used to measure sperm uptake of SYBR-14 and JC-1 fluorescent dye (Molecular Probes, Eugene, OR) specific for viability and mitochondrial potential (Graham et al., 1990; Gravance et al., 2001). The electronics of the instrument were optimized each day prior to the sacrifice of a cohort of 4-6 rats by the following procedures. 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 using CellQuest (Becton-Dickinson) and FlowJo (TreeStar) software.

For measurement of viability and mitochondrial potential, 0.5 ml aliquots of caudal sperm suspension from each rat were placed in 12x75 mm polystyrene Falcon tubes in triplicate and stained with 5 µl SYBR-14 diluted in SYBR buffer (0.15 M saline, 10% bovine serum albumin [BSA] and 10 mM HEPES), 5 µl SYBR14 plus 5 µl propidium iodide dissolved in dimethyl sulfoxide (DMSO), or 2 µl JC-1 (Molecular Probes, Eugene, OR) dissolved in DMSO, for 15 min at 37° C. Acquisitions and analyses of at least 50,000 gated events were performed as described above, and statistical analyses of fluorescent data were performed using an InStat3 software (GraphPad Software).

Measurement of cAMP

Sperm cAMP was extracted and measured according to the procedures described by Wu et al., (1995). Briefly, pre-counted sperm pellets ($1x10^7$) were re-suspended in 0.2 ml PBS and mixed with 0.8 ml ice-cold 5% trichloroacetic acid. After centrifugation at 1000 rpm for 5 min, the supernatants were acidified by adding 125 µl 1 N hydrochloric acid in 10 ml capped glass centrifuge tubes and vortexed. The samples were extracted 3 times with 2 ml ethyl ether, and the ether fraction was removed after centrifuged at 2000 rpm x 5 min. The aqueous phase after the last ether extraction was frozen and lyophilized overnight using a SpeedVac Concentrator. Pilot experiments revealed that 85-90% of ^{3}H-cAMP added to sperm suspensions or aliquots of cAMP standard solution were recovered by this procedure consistently. The lyophilized samples were re-dissolved in 150 µl 0.2 M sodium acetate (pH 4.7). Duplicates of 50 µl samples were mixed with 100 µl reaction mixture of ^{3}H-cAMP/histone /water (v/v/v=1/1/8) and 50 µl protein kinase A (142 ng/50 µl) and then incubated for 1.5 hour on ice. Thereafter, 100 µl hydroxylapatite (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 KPB and centrifuged, and

the supernatant was discarded. Subsequently, the pellet was dissolved in 100 µl of 3N hydrochloric acid by vortex, and the radioactivity was counted in 5 ml scintillation fluid with a Beckman scintillation counter. 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 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 14,000 rpm for 5 minutes, 1 µl of the supernatant of each sample was diluted in 100 µl 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 with an equal volume of 2x loading buffer (62.5 mM Tris, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, pH 6.8) and boiled at 95° for 5 minutes. Aliquots containing 20 µg proteins were then electrophoresed on a standard 10% SDS-poly acrylamide gel. Pre-stained molecular weight markers (Bio-Rad, Hercules, CA) were included in each gel. Subsequently, proteins were electro-blotted onto PVDF membrane (Bio-Rad, Hercules, CA), immuno-stained with anti-phosphotyrosine monoclonal antibody (1:1000, Santa Cruz Biotech, Santa Cruz, CA) using standard procedures. The phosphorylated protein bands were visualized using Chemiluminescence Reagent (Perkin Elmer Life Sciences, Boston, MA).

Statistics

All data were evaluated for their normal distribution, and Analysis of Variance was employed to detect effects. When the treatment effects were significant ($p<0.05$), planned *a priori* comparisons were made using 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 ($p<0.05$; $p<0.01$, Figure 1A) and inversely correlated with the height of weight drop, consistent with previous findings (Huang et al., 2003b). Unexpectedly, a significant increase in cAMP content was detected in caudal sperm of SCC rats injured by weight drop from 25-75 mm heights ($p<0.05$, Figure 1B). Cyclic AMP level of caput epididymal sperm of SCC rats, however, remained comparable to that of sham control rats. Since reduced sperm viability and mitochondrial potential were attributed to for lowered sperm motility in SCX rats (Huang et al., 2004b), we also examined sperm uptake of SYBR-14 and JC-1, fluorescent dyes specific for viability and mitochondrial potential, in SCC rats received weight drop from the 25 and 75 mm heights. As shown in Figure 1C, sperm uptake of SYBR-14 and JC-1 was significantly reduced in both groups of SCC rats ($p<0.01$).

A follow-up experiment was undertaken to determine the relationship between sperm cAMP and motility at an earlier time (4 weeks post injury). Since sperm motility was not related to the extent of cord injury at this time (Huang et al., 2003a), only rats injured by weight drop from 25 and 75 mm heights were examined. Figure 2A shows that motility of caudal sperm was significantly reduced in both groups of SCC rats ($p<0.01$). In these rats, cAMP content in caput sperm were significantly increased ($p<0.05$, Figure 2B). Cyclic cAMP content in caudal sperm of SCC rats was also increased. However, variations among animals precluded a statistical significance. In these SCC rats uptake of

SYBR-14 and JC-1 by caudal sperm were reduced significantly and equally when compared to that in sham control rats ($p<0.01$, Figure 2C).

Effects of exogenous hormones on sperm functions

To determine if cAMP-related events, viability and mitochondrial potential were also involved in hormone effects on sperm motility, these parameters were also examined in spermatozoa of SCX rats that received exogenous testosterone or FSH treatments. Eight weeks after the injury sperm motility in untreated SCX rats was significantly lower than that of untreated sham controls ($p<0.05$). 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, $p<0.05$). Similarly, TC implantation also resulted in further decreases in sperm motility in SCX rats, especially in those that received 2 cm TC implants ($p<0.01$). Sperm motility in SCX rats receiving 5 or 10 cm TC implants rebounded, but remained statistically lower in SCX rats receiving 5 cm TC when compared to that of untreated SCX rats and their sham control counterparts ($p<0.05$, $p<0.01$). Daily injection of FSH for 2 weeks also reduced sperm motility in sham control rats ($p<0.05$), and eliminated sperm motility in SCX rats ($p<0.01$).

As in SCC rats, cAMP level in caudal sperm of SCX rats was also increased despite a lack of 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 ($p<0.05$). Sperm cAMP level was also increased in SCX rats receiving 1 or 2 cm TC implant to levels that were significantly higher than that of untreated sham control rats ($p<0.05$, 0.01). 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 ($p<0.05$) 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 ($p<0.01$, Figure 4). Implantation of 10 cm TC did not affect SYBR-14 and JC-1 uptake by spermatozoa of sham control rats, but restored that in SCX rats ($p<0.01$).

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 mol wt between 93 and 115 Kd (Figure 5A). Spinal cord transection resulted in >20% decrease in this major band ($p<0.05$, 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,D). The increase in the major protein band in testosterone-treated SCX rats, when normalized against that of 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 functions 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), abnormal sperm motility seen in SCC and SCX rats cannot be ascribed to malnutrition.

Undoubtedly SCC and SCX rats were under constant stress due to lack of normal mobility. However, daily observation of stress-related indicators such as alertness, fur condition and cleanness of the eye lid, etc., indicated that the stress these animals were experiencing was minimal. These observations suggest that stress was unlikely 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 sperm motility in SCC rats suffered lesser injuries (i.e. rats received weight drop once from the 12.5 or 25 mm heights) had recovered during chronic phase of the injury, that in rats suffered more severer injuries continued to deteriorate over time (Huang et al., 2003b). The latter is consistent with that occurred in SCX rats (Huang et al., 2004a,b). We postulate that factors intrinsic to the sperm may dictate recovery or continuous deterioration of sperm motility (and perhaps other sperm functions) over time in rats suffered different extents of cord injury. Because of the importance of cAMP in various sperm functions, we sought to determine if 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., 2004b). 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 post injury was consistent with previous results (Huang et al., 2003b). Since sperm motility was suppressed to a greater extent in SCC rats that injured by weight drop from both the 25 and 75 mm heights 4 weeks after the injury, the higher sperm motility seen in SCC rats of the 25 mm group at 8 week was consistent with its recovery. Concomitant improvement of sperm viability and mitochondrial potential that exhibited better recovery in SCC rats of the 25 mm group at this time indicate that they were involved in the effects of cord injury on sperm motility. These effects could be attributed to faulty development of the sperm due to abnormal spermiogenesis or abnormal sperm maturation due to impaired epididymal function (Kempinas et al., 1998; Ricker et al., 1996).

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 since they should have been better preserved in rats by the 5 or 10 cm TC implants and had higher serum and testicular testosterone levels when compared to those that received 1 or 2 cm TC implants (Huang et al., 2004a). These effects were probably unrelated to sperm viability and/or mitochondrial potential since 10 cm TC implants did not affect sperm viability and mitochondrial function in sham control rats, but significantly improved that 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 increase was probably unrelated to hormone status in these rats since the pituitary-testis hormone axis has recovered during the chronic phase of the injury (Huang et al., 1995; 2004a). This effect was more pronounced in the sperm of SCX rats receiving 2 cm TC implant that had severely impaired motility, and was also observed in sham control rats receiving 5 or 10 cm TC implants and 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 to those rats receiving 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 down stream 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 since identical FSH treatment 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 (Rojas et al., 1993; Kopf and Vacquier, 1984; Salanova et al., 1999). The 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 were regulated by capacitation agents such as Ca++-calmodulin and bicarbonate (Kopf and Vacquier, 1984; Okamura et al., 1991; Rojas et al., 1993, Jaiswal and Conti, 2001). In addition, presence of multiple PDEs in human sperm may provide compartmentized cAMP pools for specific functions. (Fisch et al. 1998).

In the sperm cAMP activates specific protein kinases that involve 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; Adeoya-Osiguwara and Fraser, 2002). The cAMP/protein kinase A signaling events also involve in the regulation of redistribution of phospholipid and cholesterol within 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 dysfunctioning cAMP-protein kinase cascade which 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 counterpart, suggesting that 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 soluble adenylate cyclase (sAC), and sperm lacking sAC exhibited severe motility defect and was infertile (Esposito et al., 2004; Luconi et al., 2005). Understanding the effect of SCI on sperm ACs, their signaling, and downstream cellular effects may hold the key to unravel the mechanisms responsible for abnormal sperm functions after cord injury.

In conclusion, 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 suggests that sperm cAMP-protein kinase signaling events were perturbed after cord injury. While exogenous testosterone maintained sperm viability and

expressed as mean±sem of 4 rats. a: $p<0.05$ vs sham control. (C) and (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. Intensity of the major phosphorylated protein band (115> >93 kD) from each blot was scanned, and normalized against the intensity of the untreated control in each blot. Results are expressed as mean±sem of 4 blots.

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mitochondrial potential in SCX rats, lack of concomitant effect in sperm motility suggests that multiple mechanisms were involved in the effects of SCI on sperm functions.

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Legend to the figures

Figure 1. The Effect of spinal cord contusion on sperm functions 8 weeks post injury. (A) Sperm motility was reduced in all SCC rats, and these effects were inversely correlated with the height of weight drop. (B) While sperm cAMP content of caput sperm of SCC rats was comparable to that of sham control rats, that in caudal sperm in rats injured by weight drop from 25-75 mm heights was significantly higher compared to that in sham control rats. (C) The uptake of SYBR-14 and JC-1 by caudal sperm of SCC rats was significantly reduced in SCC rats injured by weight drop from both the 25 and 75 mm heights. These effects also appeared to be inversely correlated with the height of weight drop. Results are expressed as mean±sem. n= 6-8 rats per group. a: $p<0.05$, aa: $p<0.01$ vs sham controls.

Figure 2. The Effect of spinal cord contusion on sperm functions 4 weeks post injury. (A) Sperm motility was reduced in both groups of SCC rats. However, these effects were not related to the height of weight drop. (B) Cyclic AMP content in caput and caudal sperm was increased in SCC rats, but only the increase in caput sperm was significant. (C) Sperm uptake of SYBR-14 and JC-1 was equally reduced in both groups of SCC rats compared to sham control rats. Results are expressed as mean±sem. n= 6-8 rats per group. a: $p<0.05$, aa: $p<0.01$ vs sham controls.

Figure 3. The Effect of exogenous testosterone and FSH on sperm functions in SCX rats 8 weeks post injury. (A) Sperm motility was reduced in sham control rats and SCX rats receiving TC implants or FSH injections, but the extent of decrease differed significantly between two groups. (B) Sperm cAMP content in SCX rats was further increased in those received 1 or 2 cm TC implant, but was not changed in those received 5 or 10 cm TC implants. In contrast, sperm cAMP was significantly increased in sham control rats received 5 or 10 cm TC implants. FSH did not affect sperm cAMP in both sham control and SCX rats. Results are expressed as mean±sem. n = 5-7 rats per group. a: $p<0.05$, aa: $p<0.01$ vs untreated sham control; b: $p<0.05$, bb: $p<0.01$ vs untreated SCI.

Figure 4. Exogenous testosterone improved sperm uptake of SYBR-14 and JC-1 in SCX rats. (A) and (B) Representative scatter plots of sperm stained with SYBR-14 and JC-1, respectively. In sham control rat sperm uptake of both SYBR-14 and JC-1 was relatively homogenous, and was not affected by 10 cm TC implants. In SCX rat, however, intensity of the uptake of SYBR and JC-1 was heterogeneous; such effect was prevented by 10 cm TC implants. (C) Sperm uptake of SYBR-14 and JC-1 was significantly reduced in SCX rats; such effects were reversed by 10 cm TC implants. Results were presented as mean±sem of 6 rats. aa: $p<0.01$ vs untreated sham control, bb: $p<0.01$ vs untreated SCX.

Figure 5. Effects of SCX and exogenous testosterone 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 the intensity of the major phosphorylated sperm protein between sham control and SCX rats. Results are

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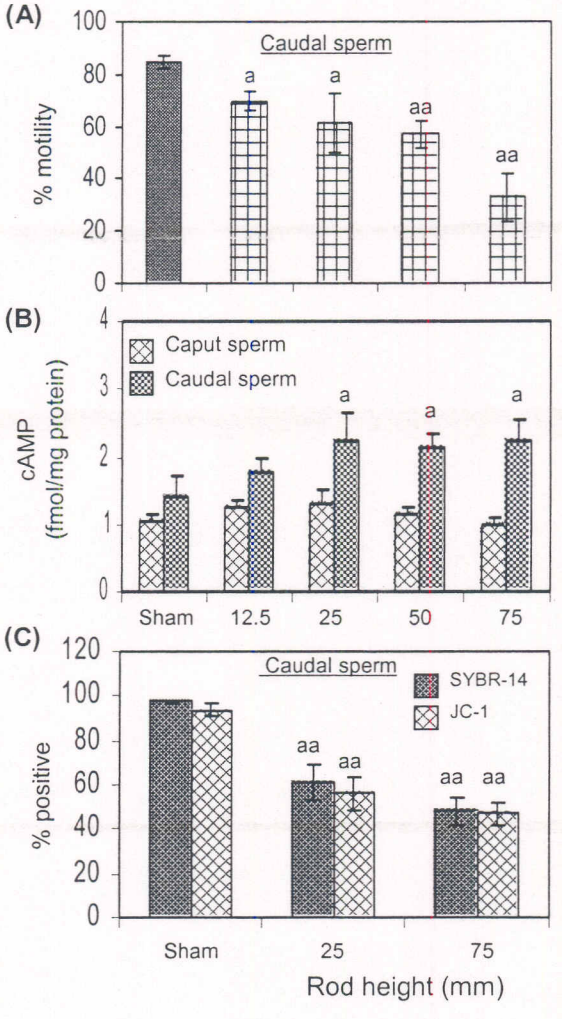
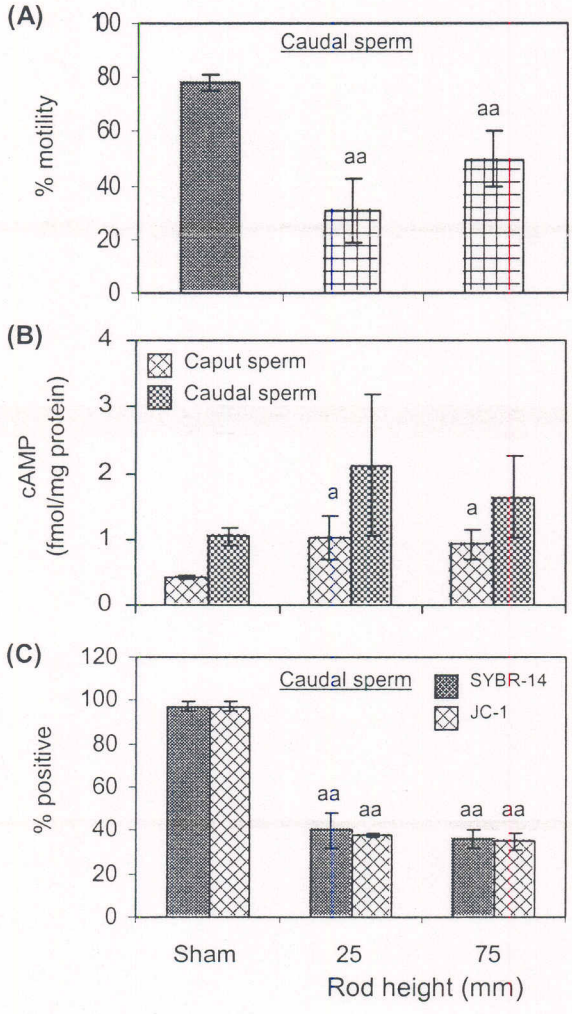


Wang et al. Figure 2



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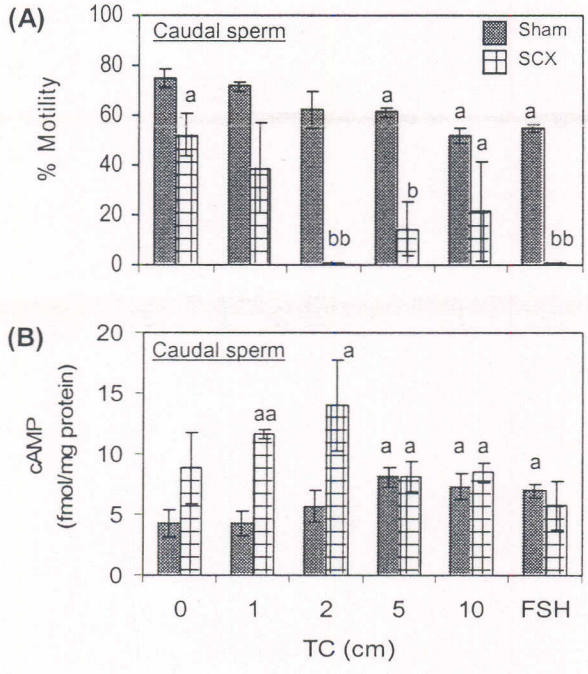

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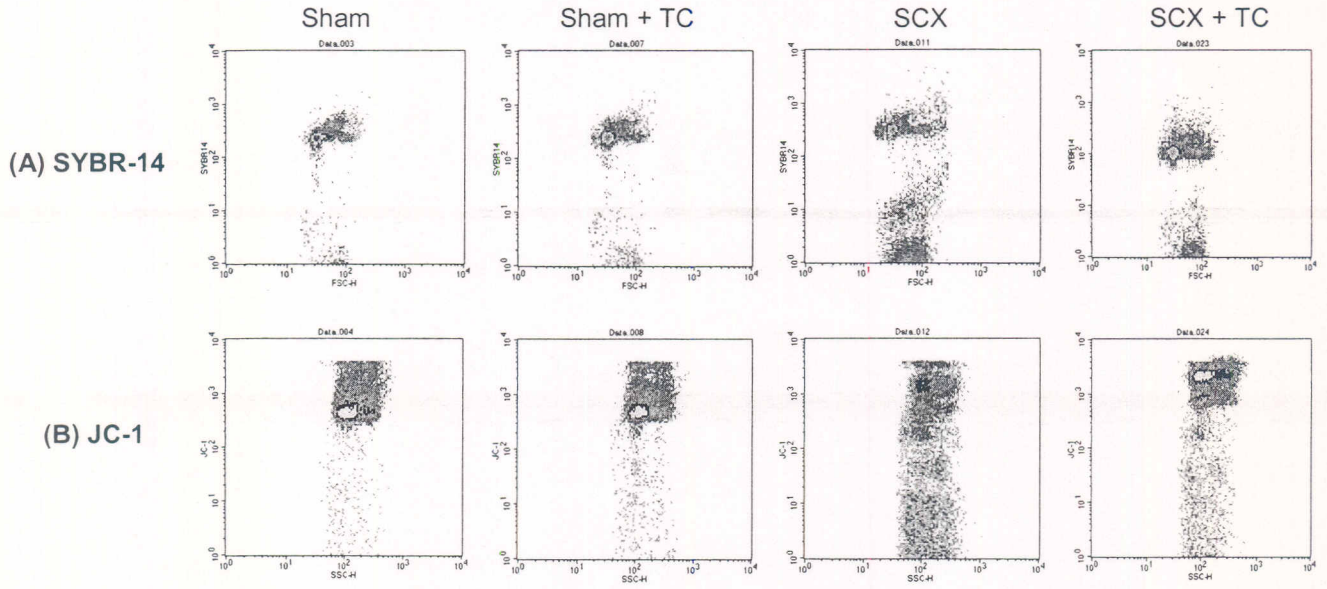

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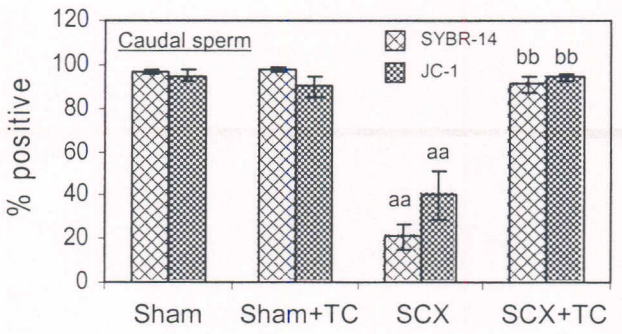

Wang et al. Figure 5