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A New Method to Generate Canine Seminal Emission and Its Application to Men: Direct Electrical Stimulation of the Vas Deferens

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ABSTRACT: Seminal emission from the ejaculatory duct (SEED) by direct electrical stimulation of the vas deferens was investigated in the dog, and the technique was applied to men. The stimulus parameters used were 2 msec, 10 Hz, and 8 V for dogs or 15–20 V for humans. *In vitro* studies using tetrodotoxin demonstrated that the major portion of the muscle contraction under the above stimulation was neurogenic. The stimulation of the pars epididymica, the middle vas, or the ampulla of the vas caused SEED in all dogs having intact hypogastric nerves (HNs) and receiving transection of bilateral HNs 1, 6, and 12 months before electrical stimulation. The dye instilled into the canine cauda epididymis was transported to the ampulla and emitted into the posterior urethra by electrical stimulation of the vas regardless of the site stimulated. The electrical stimulation of eight vasa deferentia (pars epididymica) of five prostatic carcinoma patients generated emission from the severed proximal end of all vasa examined at orchidectomy. All of the stimulations of 13 middle vasa of seven patients with emission loss caused SEED. The above results indicate that direct electrical stimulation of the canine and human vas deferens causes SEED regardless of the site stimulated or the absence of HNs, which are the major pathway of the efferent signal for SEED.

Key words: Spermatozoa, artificial emission, vas stimulation.
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Seminal emission from the ejaculatory duct (SEED) is regarded to be a spinal reflex that is further controlled by the upper central nervous system. Loss of SEED is a cause of infertility in men with spinal cord or other nerve injuries. Artificial seminal emission by stimulating a site of the above reflex arch has been tried in such patients, including subarachnoid injection of anticholine esterase (Guttman and Walsh, 1971), direct electrical stimulation of the hypogastric nerve (Brindley et al, 1989), transrectal electrical stimulation of the pelvic plexus or prostatic plexus (Horne et al, 1948; Ohl et al, 1991), and vibration of the penis (Brindley, 1984). However, success of artificial emission by these methods has been limited because of serious side effects, low effectiveness, necessity of invasive operation, and/or necessity of general anesthesia. Despite the recent development of methods to induce artificial erection (Small et al, 1975; Virag, 1982), effective methods for inducing ejaculation are lacking.

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It has recently been found that direct electrical stimulation of the cauda epididymis that stores spermatozoa generates SEED (Sato et al, 1991). The objective of the current study was to attempt induction of SEED using direct electrical stimulation of the vas deferens. The method was validated first in dogs, where SEED could be induced after bilateral hypogastric nerve transections. Experiments were conducted to demonstrate electrically induced contraction of the vas deferens and that this contraction was neurogenic in nature. Finally, this method was applied to men with and without emission loss.

Materials and Methods

Animals

Adult male mongrel dogs weighing 15–20 kg were used. The operations were carried out under anesthesia with ketamine hydrochloride (10 mg/kg body weight) and pentobarbital sodium (5 mg/kg body weight).

Men

Seven patients; including six by spinal injury and one by previous retroperitoneal lymph node dissection, were selected as the emission loss group for the present study. Five patients capable of

emission with prostatic carcinoma (D2 stage) were selected as the emission group, with informed consent.

Electrical Stimulation of the Canine Vas Deferens

Electrical stimulation was applied to the vas deferens directly under vision with two wire electrodes (1 cm apart) after exposure of the whole vas by inguinal and abdominal incisions. The wire part of the electrode-needle for nerve block (Pole needle; TOP Corporation, Tokyo) was used as the wire electrode. Square pulses of 8 V, 2 msec duration, and 10 Hz were provided by a stimulator (model DPS-06; Daiya Medical System Corporation, Tokyo) and applied to the pars epididymica or the middle or ampulla of the vas. Middle vas refers to the portion between the pars epididymica and the ampulla in the current study. The stimulation was applied for 5 minutes or until SEED, whichever occurred first. The vas deferens was stimulated either in the presence of intact bilateral hypogastric nerves or 1, 6, and 12 months after their transection (Kihara et al, 1992a). The stimulation was limited to once for each vas.

Electrical Stimulation of the Human Vas Deferens

At orchidectomy for the emission group, the pars epididymica of the vas was exposed under anesthesia with 1% lidocaine and stimulated directly with the two wire electrodes as described above. For emission loss patients, the middle vas was stimulated directly by penetrating through the scrotal skin with two Pole needles (1 cm apart) while manually stabilizing the middle vas. Stimulus parameters for men were the same as those for dogs except for the voltage, which was increased to 15–20 V.

Observation of Seminal Emission

In the dog, SEED was observed as previously described (Kihara et al, 1991). Briefly, the verumontanum was exposed under vision through a ventral midline incision on the prostate, and bladder neck and bilateral ureters were ligated to interrupt urine flow for accurate observation of SEED. Before stimulation, the emptiness of the ampulla was confirmed by the absence of SEED during the procedure of pressing the ampulla against the prostate with a finger five times. SEED was considered to have occurred by the stimulation when semen flowed into the posterior urethra, either spontaneously or after manual compression of the ampulla. The emitted seminal fluid was collected with a Pasteur pipette and put into 50 ml of 5% glucose for microscopic determination of numbers of spermatozoa. To evaluate motility, a drop of seminal fluid diluted with 5% glucose was put on a slide glass and covered with a cover glass. Motility rate was estimated in five fields. Motile spermatozoa included those showing either any progressive forward movement or flagellar movement.

In humans, the posterior urethra was washed with 50 ml of 5% glucose after electrical stimulation. The number of spermatozoa was determined microscopically in five fields using the above washed fluid within 10 minutes of discontinuing stimulation, and their movement was observed simultaneously, as described in the canine experiment. Before electrical stimulation, emptiness of the ampulla was confirmed by observing no spermatozoa in the sediment of the 5% glucose solution with which the posterior urethra was washed following transrectal compression of the ampulla (five times) with a finger. If any spermatozoa were present in the above fluid, the pressing was repeated until no spermatozoa were observed. At orchidectomy, the cauda epididymis was directly stimulated by the electrodes, and the presence or absence of seminal flow from the proximal end of the severed vas was determined. The number of spermatozoa in the seminal fluid was counted microscopically after dilution with 10 ml of 5% glucose. The presence or absence of motile spermatozoa was noted without estimation of their ratio.

Observation of the Movement of Dye Instilled into the Canine Cauda Epididymis and Pars Epididymica of the Vas Deferens

Indigo carmine (0.04 ml of a 0.4% solution) was instilled into the lumina of the cauda epididymis and pars epididymica of the vas through the distal end of the pars epididymica with a 27-G needle. The pars epididymica and the middle and ampulla of the vas were each given a single stimulation, and movement of the dye was observed.

Evaluation of the Stimulus Parameter and Its Dependence upon Nervous Transmission

Contraction of the vas was measured *in vitro* as previously described (Morita et al, 1993). Briefly, a 2-cm-long segment of the vas (ampulla) was obtained from each of three dogs and mounted in a 20-ml bath at 37°C containing Krebs' solution gassed with 95% O_2 and 5% CO_2. One end of the duct was anchored with a 4-0 silk thread to a fixed hook in the bottom of the bath, and the other end was similarly anchored to a Statham UC-2 force transducer. A baseline force of 1.0 g was applied and maintained during the experiment. The duct was electrically stimulated with the parameters of 0.1–10 msec duration, 80 V, and 20 Hz for 5 seconds. Tetrodotoxin (3×10^{-7} M) which inhibits nerve-induced muscle contraction (Hashimoto et al, 1967), was added during the electrical stimulation.

Results

Evaluation of the Stimulus Parameter: Stimulation of the Nerve or Muscle?

As shown in Figure 1, tetrodotoxin (3×10^{-7} M) markedly inhibited the contraction of the canine ampulla due to stimulation of 2 msec duration, indicating that the major portion of the vas contraction due to stimulation was neurogenic.

SEED by Electrical Stimulation of the Vas Deferens of Dogs Before or After Transection of Bilateral Hypogastric Nerves

The results are summarized in Table 1. The stimulation of the pars epididymica, the middle vas, or the ampulla caused SEED regardless of the presence or absence of bilateral hypogastric nerves. The number of spermatozoa obtained was $5.2 \pm 2.3 \times 10^7$, of which 58 ± 11% were motile and 18 ± 13% showed progressive forward movement.

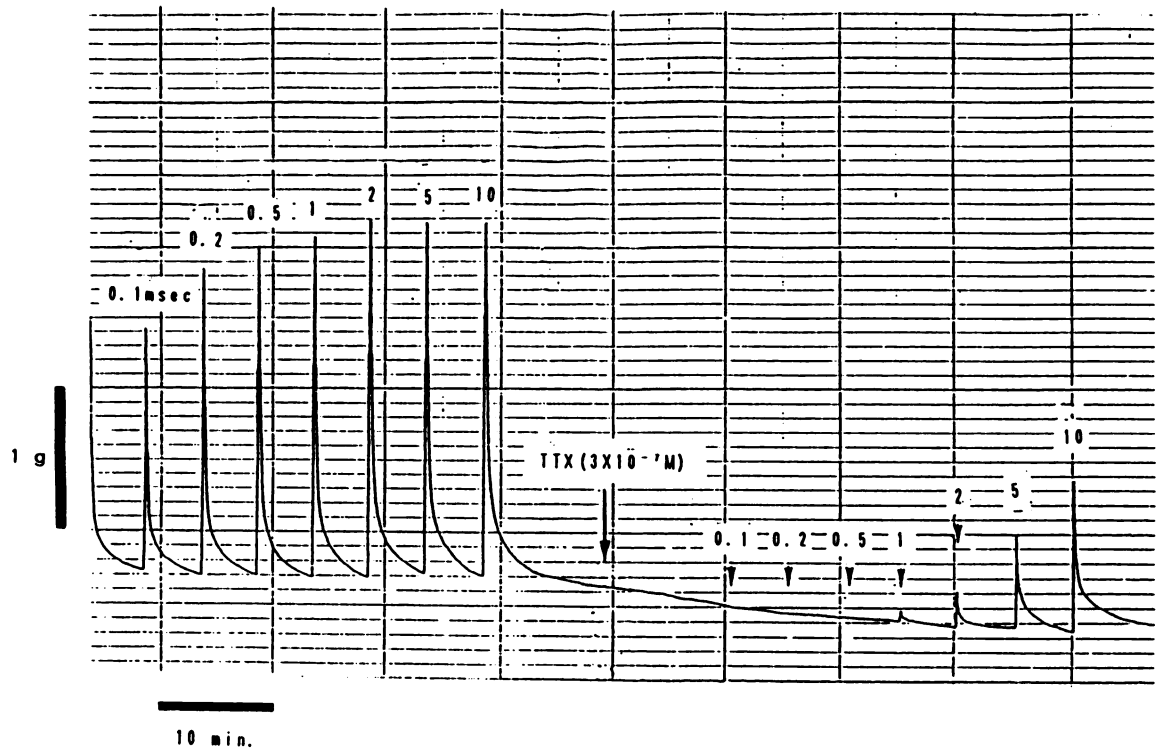

FIG. 1. Inhibition of muscle contraction of the ampulla of the canine vas deferens by tetrodotoxin. Stimulus parameters for electrical stimulation of the vas were 0.1–10 msec duration, 80 V, and 20 Hz for 5 seconds. The arrow indicates addition of tetrodotoxin at the concentration of 3×10^{-7} M. See the Materials and Methods section for further details.

Movement of the Dye Instilled into the Cauda Epididymis and Pars Epididymica of the Vas by Electrical Stimulation of the Canine Vas Deferens

Regardless of the site stimulated, a major portion of the dye in the cauda epididymis was transported to the ampulla, and some of the transported dye was secreted into the posterior urethra (Table 2).

Emission from the Proximal End of the Severed Vas by Electrical Stimulation of the Human Pars Epididymica

Eight vasa deferentia of five patients with prostatic carcinoma were examined at the opportunity of orchidectomy. Electrical stimulation directly applied to the pars epididymica caused emission from the proximal end of the severed vas at the level of the external inguinal ring in all cases examined (Table 3). In each experiment, one to three drops of seminal fluid were obtained that contained more than 1×10^7 spermatozoa, including many spermatozoa with forward movement, but their motility rate was not estimated.

Table 1. *Seminal emission by electrical stimulation of the vas deferens of dogs with intact or transected bilateral hypogastric nerves*

Number of months before transection of bilateral hypogastric nerves	Stimulation sites of the vas deferens		
	Pars epididymica	Middle	Ampulla
None (no transection was performed)	6/6*	6(2)/6	6(1)/6
1	6(1)/6	6(1)/6	6(2)/6
6	6(2)/6	6(2)/6	6(3)/6
12	6(2)/6	6(3)/6	6(3)/6

* Values represent cases of seminal emission/number of dogs examined. Numbers in parentheses show cases of seminal emission confirmed by pressing the ampulla.

SEED by Electrical Stimulation of the Vas Deferens of Men with Emission Loss

The results are summarized in Table 4. All stimulations of 13 middle vasa of seven patients with emission loss generated SEED, although pressing of the ampulla was necessary for each case examined. More than 2×10^7 spermatozoa were obtained in all trials except for two (cases 4 and 5), but the motility rate, including flagellar and progressive movement, was low in all ejaculates obtained. No major side effects were observed except for a slight pain at the stimulating site of the scrotum during stimulation in some patients.

Discussion

The present results indicate that electrical stimulation of the vas deferens causes SEED regardless of the site stim-

Table 2. *Transportation of indigo carmine in the canine cauda epididymis by electrical stimulation of the vas deferens*

Transportation of the dye	Stimulation sites of the vas deferens		
	Pars epididymica	Middle	Ampulla
Disappearance of a major portion from the cauda epididymis	6/6*	6/6	6/6
Entry into the ampulla	6/6	6/6	6/6
Emission to the posterior urethra	6(2)/6	6(1)/6	6(2)/6

* Positive cases/numbers of vasa deferentia examined. See footnote to Table 1 for other details.

Table 3. *Emission from the proximal end of the severed vas by electrical stimulation of the pars epididymica of the human vas deferens*

Case	Age	Side	Number of sperm
1	62	R	$>1 \times 10^7$
		L	NE
2	79	R	$>1 \times 10^7$
		L	NE
3	68	R	$>1 \times 10^7$
		L	$>1 \times 10^7$
4	72	R	$>1 \times 10^7$
		L	$>1 \times 10^7$
5	77	R	$>1 \times 10^7$
		L	$>1 \times 10^7$

R, right; L, left; NE, not examined. Numbers $>1 \times 10^7$ were not determined.

ulated. Seminal emission is a phenomenon caused by a spinal reflex; the afferent signal of this reflex passes through the penile dorsal nerve and the pudendal nerve, while the main efferent signal reaches to the vas deferens through the lumbar sympathetic trunk, the splanchnic nerve, the superior hypogastric plexus, the hypogastric nerve, and the pelvic plexus (Kihara et al, 1992b, 1993). In contrast to previous trials for artificial seminal emission that have depended upon the existence of the above reflex arch or at least the pelvic plexus (Horne et al, 1948; Guttman and Walsh, 1971; Brindley, 1984; Ohl et al, 1991), the present method, which stimulates the effective organ itself, is applicable to emission loss patients lacking the reflex arch or the pelvic plexus. The current study has further demonstrated that there are no specific sites for electrical stimulation to cause SEED in the dog and humans. The vas deferens itself can be easily identified and held over the scrotal skin using the fingers. Direct stimulation of the vas using needles is easy, safe, and repeatable.

Stimulation of the vas of the dog caused spontaneous SEED regardless of both the site stimulated and the absence of hypogastric nerves. Indeed, electrical stimulation of the vas of men with long-term emission loss has induced transportation of seminal fluid to the ampulla but did not induce spontaneous SEED. Further, the number and motility of spermatozoa obtained were in low levels. Functional and morphological changes of the vas and reduced sperm motility have been reported after transection of inferior mesenteric plexus, from which bilateral hypogastric nerves originate in the rat (Billups et al, 1990). The current discrepancies between men with long-term emission loss and dogs receiving prior transection of bilateral hypogastric nerves are partially attributable to either the difference of the species or the difference in the effectiveness of stimulation by the electrode to the vas under direct vision and guiding by the finger. Although a diluent (5% glucose) might also influence motility of the spermatozoa in men with emission loss, application of the complex medium M-199 to SEED of a patient (case 1) failed to improve the motility of the spermatozoa (data not shown), and motile spermatozoa were present in the fluid from the vas of cancer patients that was diluted with 5% glucose solution. Because the nerve system affects the nutrition of the organ under its control, the discrepancy may also depend on the difference in the extent of the injury to the sympathetic nervous system controlling the vas deferens. Recently, two compensatory pathways were demonstrated to generate SEED after bilateral hypogastric nerve transections; one is via the lumbosacral sympathetic trunk and the pelvic nerve (Kihara et al, 1991), and the

Table 4. *Seminal emission by electrical stimulation of the vas deferens of men with emission loss*

Case	Age	No. of trials	No. of sperm	Motility (%)	Cause of emission loss
1	31	5	6.3×10^7	3	RPLND
			5.9×10^7	<1	
			9.6×10^{7}*	<1	
			6.3×10^7	3	
			8.6×10^{7}*	1	
2	36	2	3.0×10^6	<1	SI
			2.0×10^6	<1	
3	28	2	3.1×10^7	<1	SI
			1.0×10^{8}*	<1	
4	41	1	3.3×10^6	<1	SI
5	24	1	2.1×10^6	<1	SI
6	34	1	2.3×10^7	1	SI
7	33	1	2.6×10^7	3	SI

Seminal emission occurred by pressing the ampulla with a finger transrectally after electrical stimulation in each case. Emptiness of the ampulla before stimulation was confirmed as mentioned in the Materials and Methods section.

* These trials showed spermatozoa in the sediment of the urine obtained just after the pressing before electrical stimulation of the vas, but the number was less than 10% of that obtained following electrical stimulation. RPLND, retroperitoneal lymph node dissection; SI, spinal cord injury.

other is via the spermatic nerve (Sato et al, 1991). The dogs whose bilateral hypogastric nerves had been transected showed retrograde ejaculation by manual penile stimulation and SEED by electrical stimulation of either of the compensatory pathways (Kihara et al, 1991, 1992a). It could be assumed that all three of these pathways were damaged in the men with emission loss examined in the current study. The nutrition system of their seminal tracts may have been impaired, leading to dysfunction of the seminal tract and stasis of spermatozoa in the epididymis that could result in degenerative damage to the spermatozoa. The very poor quality of spermatozoa obtained by SEED makes its use for artificial insemination (AI) or *in vitro* fertilization (IVF) questionable, but it might be possible to use such spermatozoa for the recently developed technique of intracytoplasmic egg injection (ICSI).

Transportation of the content of the cauda epididymis in the distal direction occurred due to stimulation of the vas deferens regardless of the site stimulated. According to the current hypothesis (Ventura et al, 1973; Kimura et al, 1975), spermatozoa are transported from the cauda epididymis to the ampulla with the nerve-induced muscle contraction that starts at the cauda epididymis and gradually moves to the distal vas. If so, muscle contraction at the stimulated site might interrupt seminal flow from the cauda epididymis. Our current study reveals the insufficiency of the above hypothesis.

The current study introduced a method of causing seminal emission from the ejaculatory duct in the dog that was applicable to patients with emission loss as a safe and accurate method to obtain spermatozoa. Further, it provided additional information on the mechanism of sperm transport through the vas deferens.

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