

EFFECT OF BUTHUS OCCITANUS TUNETANUS ENVENOMATION ON RAT PARTURITION

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SUMMARY:

The aim of this work was to evaluate the scorpion envenomation effects on parturition process of rats. Four groups of pregnant rats were used. The two first-groups received a sub-lethal single dose of crude venom of *Buthus occitanus tunetanus* (bot) at the 22nd day of pregnancy by intraperitoneal (i.p.) injection. Group I was left until delivery, and served to notify intoxication symptoms related to pregnancy. The group II was used for in vivo electromyographic study of uterine contractility. Group III and IV, had received a saline solution at 0.9 % NaCl and served as controls for group I and II. At the end of electromyographic recording (group II and IV), uterine tissues were prepared for light microscopy observation.

Group I manifested a vaginal bleeding after the venom injection. Their foetal delivery was delayed compared to controls (group III). The electromyographic study revealed that the number and frequency of bursts were significantly increased in envenomed rats. Few oedema and fiber dissociation were observed in uterine myometrium of envenomed rats.

It was concluded that, scorpion bot envenomation may induce a dynamic dystocia by disturbing the uterine motility in pregnant rats.

Key words: scorpion – venom – Rat- pregnancy.

INTRODUCTION :

Scorpion sting, present a public health threatening in many regions in the world. Calderon- Aranda (1993) reported that about 250.000 scorpion human sting occurred each year in Mexico. In Tunisia 35.000 to 40.000 human scorpion poisoning were recorded each year [El Ayeb et al (1994)]. The scorpion poisoning vulnerability belongs to numerous neurotoxins actions on ion channels activity of excitable cells especially neuronal and muscular cells. Many in vitro studies confirmed that neurotoxins enhance the myometrial motility [Mendonça et al (1995), Osman et al (1972) and Marei et Ibrahim (1979)], of different female uteri. Thus, it is suggested that scorpion envenomation in pregnant female may influence the parturation and labour process. This work is the first experimental study describing the scorpion envenomation effects on rat parturition and uterine electromyographic activity.

MATERIALS AND METHODS:

Animals: 3 to 4 month-old virgin female rats were obtained from Pasteur Institute of Tunis and transferred to the animal room of Pharmacology

department of the Faculty of Medicine of Sfax. Animal room condition was fixed at 20 to 25°C temperature and 14H00 light and 10H00 darkness diurnal cycle. Water and nutrition were given ad libitum. Female rats were left with mature males, from 18H00 to 09H00 of the next day, to allow the copulation. The day on which spermatozoa were detected in the vaginal plugs was considered as the first day of pregnancy.

Scorpion venom : the scorpion crude *bot* venom was obtained from the Pasteur Institute of Tunis. It was prepared by the electrical milking of *bot* scorpions; water extracted [(Miranda et al (1970)], freeze-dried and conserved at -20 °C. The crude scorpion venom was diluted by a solution of a physiological saline at 0,9 % of NaCl to obtain venom concentration of 500µg proteins/ml.

Groups:

Four groups of pregnant rats were used with six pregnant rats for each group.

The two first-groups received a sub-lethal single dose of crude venom of 500µg / ml / Kg of *bot* at the 22nd day of pregnancy by i.p. Injection.

Group I was left until delivery, and served to notify the effect of the scorpion envenomation on parturition.

Group II was used for in vivo electromyographic study of uterine contractility.

Group III and IV, received 1 ml / Kg (BW) physiological saline solution at 0.9 % NaCl and served as controls for group I and II.

Effect of the scorpion envenomation on parturition:

To determine the effect of the *bot* envenomation on the time of parturition, we estimated the time elapsed for delivery to start after injection of the venom or saline solution (between the latest treatment done from 09H00 to 09H05 of the 22nd day of pregnancy and the first foetus delivery).

The labour time (time separating the first and the last delivered pup) was measured and the foetal delivery duration was estimated by: labour time (min) / number of neonatal rats.

Effect of scorpion envenomation in uterine electromyographic activity:

Uterine electromyographic activity was carried out dealing with the method described by Demianczuk et al (1984). It was achieved on pentobarbital sodium anaesthetized rats (60 mg /ml / kg). Rats were artificially ventilated using a pressure controlled respirator –1002 and allowed to stabilize for 10 min. Venom or saline solution were then injected intra peritoneally on the animal back. An abdominal incision of about 2 cm length was made, for the in vivo electromyographic activity measurements. Two bipolar electrodes were implanted in the external layer of uterine myometrium, separated by 0.5cm length, in a non gravid(uterine region without placental attachment) median region of uterine horn. They served to detect the electromyographic bursts. A ground one was inserted in the abdominal wall. Electromyographs were recorded by a PRAXIGRAPH ALVAR (PARIS)II. Only bursts and quiescent periods with a duration over 4 cm (15 s) length were measured using a rule. Electro-myographic burst amplitudes upper than 0.6 cm (100 μ V) were recorded and their mean values for each burst wave were calculated each 10 min.

Light microscopy observation:

After the electro-myographic recording, uterine tissues were excised from non placental sites of the median uterine region, fixed in a 10% formaldehyde solution , incubated in a series of formol, alcohol, toluene and paraffin; using an

automate (Histochnette Shanon Citadel 2000) and embedded into paraffin.

6-8 μ m tissue sections were de-waxed, re-hydrated and stained with hematoxylin for light microscopic observation.

Statistical analysis:

All results were expressed as mean \pm SEM. ANOVA (A) analysis and Kruskal- Wallis test (K) were used to compare the envenomed rat parameters with control ones. Significance of differences was considered when $P \leq 5\%$.

RESULTS :

Effect of the scorpion envenomation on parturition

All envenomed rats exhibited intoxication symptoms as mouth rubbing, mastication, des-equilibrium, paralysis, salivation, agitation, polypnea and squeaking. A vaginal bleeding without pup delivery was observed in *bot* rats and not in their controls (table I).

Table I: Experimental observation of the effect of scorpion envenomation on pregnant rats:

treatment	Vaginal bleeding without pup delivery	
	Number of rats	Timing (min)
0,9 % NaCl	0	--
500 μ g / ml / Kg Bot	2	148 154

Timing: the time occurring between the latest treatment and vaginal bleeding.

The elapsed time for delivery to start after injection of saline or venom was significantly higher in envenomed rats than in their controls($P= 0,00394$) (fig.1).

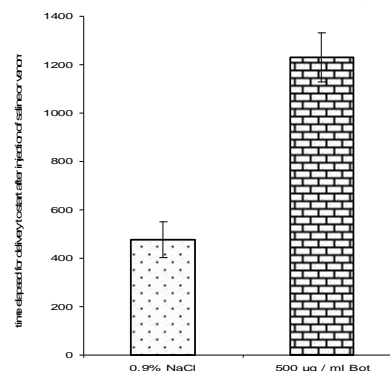


Fig. 1: effect of the scorpion envenomation on parturition

Bot: Buthus occitanus tunetanus crude venom; The elapsed time for delivery to start after injection of saline or venom was significantly higher in envenomed rats than in their controls(Kruskal-Wallis test, $P= 0,00394$).

The estimated foetal labour time had increased significantly in envenomed rats in comparison with their controls ($p = 0.000066$)(fig.2).

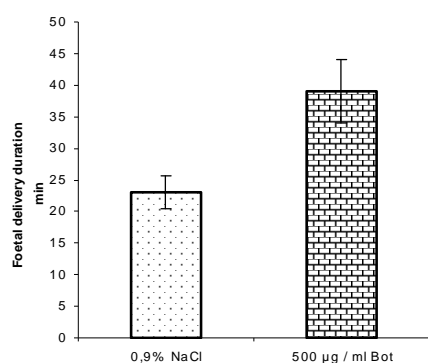


Fig.2: effect of the scorpion envenomation on the foetal delivery duration

Bot: *Buthus occitanus tunetanus* crude venom; The estimated foetal labour time had increased significantly in envenomed rats in comparison with their controls (Kruskal-Wallis, $p = 0.000066$).

Table II : Electromyographic measurement of the uterine activity

Time intervals of measurements (min)	Treatments	Electromyographic bursts number		Electromyographic bursts amplitudes		Electromyographic bursts duration		Quiescent intervals	
		mean	(±SD)	mean	(±SD)	mean	(+ SD)	mean	(±SD)
[0 – 10]	Bot	5.8	(±1.47)	15.1	(±4.0)	1.83	(±0.29)	67	(±8)
	Saline	3.8	(±2.40)	13.4	(±9.8)	1.22	(±0.35)	108	(±19)
	P value	0.011 *		0.015 *		0.009 *		0.0005 *	
[10 – 20]	Bot	5.7	(±1.21)	14.7	(±4.7)	1.81	(±0.26)	68	(±32)
	Saline	2.2	(±2.04)	10.2	(±7.7)	0.89	(±0.51)	126	(±20)
	P value	0.004 *		0.307		0.002 *		0.003 *	
[20 – 30]	Bot	6.3	(±1.97)	13.7	(±8.5)	1.62	(±0.38)	70	(±33)
	Saline	1.3	(±1.63)	7.2	(±12.0)	0.62	(±0.81)	133	(±25)
	P value	0.0007 *		0.254		0.0021 *		0.003 *	
[30 – 40]	Bot	5.3	(±1.97)	18.7	(±7.7)	1.58	(±0.37)	61	(±27)
	Saline	2.7	(±2.66)	6.2	(±7.1)	0.78	(±0.67)	120	(±33)
	P value	0.00001 *		0.706		0.029 *		0.007 *	

* P < 5% (significant differences)

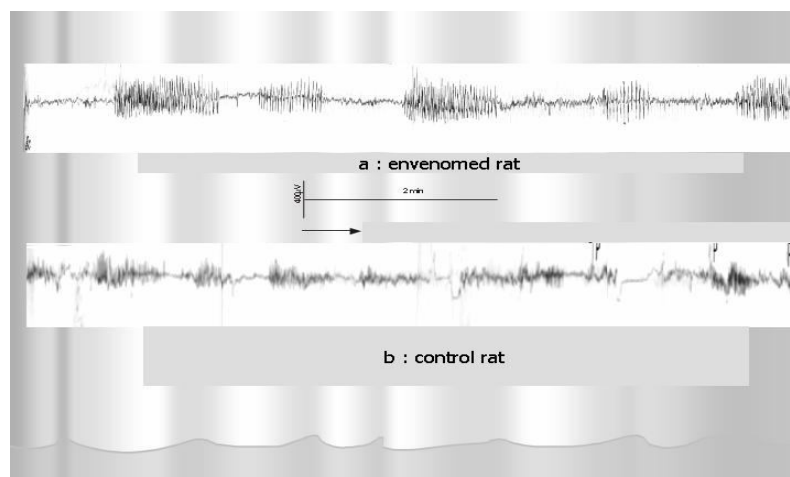


Fig.3 : Uterine electromyographic activity at the 22nd day of pregnancy after venom (a) and saline (b) injection.

Light microscopy observation:

No necrosis, nor lesions were observed in uterine myometrial tissues, in both envenomed and control rats. A stromal oedema and cell dissociation were notified at 40 min after venom injection, but not after saline injection.

DISCUSSION:

Our study is the first that evaluate the scorpion envenomation effects on rat parturition and uterine electromyographic activity.

Our study show that the envenomation by *bot* may trigger labour in late pregnant rats, manifested by the vaginal bleeding. This result was approved by uterine electromyographic activity of envenomed rats. Indeed, envenomation by *bot* increased the electromyographic bursts number and amplitude and decreased the silent periods.

Venom morbidity was said to be due to neurotoxins contained in the venom. Such components may disturb the function of the excitable cells by modifying their membrane potentials. The scorpion envenomation may also participate in the increase of the pregnant rat uterine motility by an indirect pathway. In fact, it was reported that a potentiating-bradykinin fraction, isolated from the *bot*, induced the increase of the plasma levels of estradiol [Nassar et al (1990)] and stimulated the prostaglandins and their metabolite biosynthesis, such as the inducible prostaglandin-F₂alpha [Abdel Raheim et al (1997)]. Estradiol [Juarez- Bengoa et Perusquia (1997)] and prostaglandin-F₂ alpha [Oropeza et al (2002)] play an important role in the up-regulation of the uterus contractility during the course of gestation.

Such findings suggest that the scorpion envenomation by *bot* increases the uterine contractility of pregnant rat and may induce a preterm labour. However, we found that envenomation delayed parturition and prolonged delivery and labour time. Such results are similar to those observed in the case of dynamic dystocia in pregnant females. Stromal oedema observed in light microscopy approve this idea. Dynamic dystocia causing labour failure is commonly known to be due to abnormalities in uterine contractions. In conclusion, scorpion *bot* envenomation may induce a dynamic dystocia by disturbing the uterine motility in pregnant rats. This dystocia is objectived by the uterine electromyographic hyperactivity, the delayed parturition and the prolonged labour time.

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