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INDUCTION OF GAMETOGENESIS IN THE GONADS OF GAMBUSIA AFFINIS HOLBROOKII BY THE INJECTION OF A FRACTION OF SCORPION VENOM

(With 2 Tables & 4 Fig.)

By

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تحفيز عملية تكوين كل من الحيوانات المنويه والبويضات بتأثير مستخلص مفصول من سم العقرب على مناسل سمكة الجامبوزيا أفينيس هولبروكاى

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فى هذا البحث تم دراسة تأثير مفصول من سم العقرب بوثاس أوكسى تاناس على مدى نضج بويضات انثى سمكة الجامبوزيا وعملية تكوين الحيوانات المنويه لذكور نفس السمكه فى فترة كمونها .

ولقد تبين بعد ٢٤ ساعه من حقن الاسماك بجرعه غير مميته من هذا المفصول غياب تام للبويضات المنحله بينما زاد عدد وحجم البويضات الصغيره . ورغم عدم وجود بويضات ناضجه فى هذه الفتره فى الاسماك العاديه الا انه لوحظ وجودها فى الاسماك المحقونه (المعامله).

أما فى حالة الذكور فقد تبين ان هذا المفصول ادى الى نقص عدد حاملات الحيوانات المنويه الغير ناضجه وزيادة معنويه فى عدد حاملات الحيوانات المنويه الناضجه .

وترجع هذه التأثيرات الى فعل هذا المفصول فى تنشيط البراديكينين الموجود فى هذه الاعضاء أو تأثير غير مباشر عن طريق تنشيط عملية تكوين البروستاجلاندين .

SUMMARY

The effect of extracted peptide factor with bradykinin potentiating activity from venom of *Buthus occitanus* on oocyte maturation and spermiation were studied in adult female and male *Gambusia affinis* holbrookii fish during the resting stage. Twenty four hours after the injection of a single sublethal dose (1 ug/g) of this extracted factor, the degenerating ovocytes in the female fish were completely absent, the number and size of the young ovocytes were increased and mature ovocytes were observed in the treated fish but none in the control fish. In adult male Gambusia, the injection of this factor caused a decrease in the number of immature spermatophores to produce a significant increase in the number of mature ones. This biological effect was basically interpreted to the direct initiating mechanism of such a venom factor or endogenously activated bradykinin in these organs or indirect effect through the activation of prostaglandin synthesis.

Keywords: Induction gametogenesis, gorads, Gambusia affinis holbrookii, injection, scorpion venom

INTRODUCTION

Bradykinin is a typical plasmakinin that cause contraction of isolated smooth muscle, vasodilation and increased permeability of capillaries *(CHERRY* et al., *1982)*. Bradykinin has been described as a potent mitogen in various fibroblastic cell lines (Goldstein and Wall, 1984). Marceau and Tremblay (1986), reported that bradykinin enhances cell growth. The kallikrein-kinin system seems to have stimulatory effects on the reproductive function., and all components of the kinin system are present in male and female genital secretions. Rohen and Stuttmann (1975) declared that kallikein treatment of premature rats led to the first appearance of A-spermatogonia (1-2 days earlier). It increases ^{3}H-thymidine incorporation in the DNA of the testicular tissue of adult rats (Mathiessen and Rohen, 1975). Kallikrein increases also testicular blood flow *(BLUME* et al., *1975)*, activates the sertoli cell function of testis (Rohen and Buschuter, 1975) and increases the number of supporting cells *(KLEEBERG* et al., *1975)*. Rohen and Stuttmann

(1975) found that kallikrein increases the number of spermatocytes of rats.

On the other hand, Espey (1980) and Smith and Perks (1983) reported that bradykinin may be involved in the ovulatory process. The ovarian kinin-generating activity increases significantly during ovulation *(ESPEY* et al., *1986). Yoshimura* et al., *(1988)* indicated that it induces ovulation in perfused rabbit ovaries.

OSHIMA et al., *(1969)* found that kinin is released by a kininogenase isolated from various kinds of snake venoms. Mohamed and khaled (1969) interpreted the hypotensive effect of *Cerastes cerastes* venom to result from a kinin peptide present in a free form in the venom or resulting from kinin releasing enzyme that activates the kinin from its inactive precursors in plasma .

Ferreira (1965)declared that the venom af snake; Bothrops *Jararaca,* contains an alcohol soluble fraction which potentiates the effect of bradykinin both *in vivo and vitro.* This fraction was called bradykinin potentiating factor (BPF). Isolation of bradykinin potentiating factors from different-species of snake venoms became a focus interest within the field of toxicology. BPF has been recognized in venoms of snakes; *Bothrops jararaca (SUZUKI* et al., *1967) , Agkistrodon halys blomohoffii* (Kato and Suzuki, 1970), Egyptian cobra, *Naja haje* and scorpions, *Buthus occitanus and Leiurus quinqueste-riatus (NASSAR* et al ., *1989).*

Abd El-Rehim (1990) found that an extracted factor with bradykinin potentiating activity (BPF) isolated from the scorpion venom of *Buthus occitanus* has a marked potentiating activity upon the process of spermatogenesis of premature male amd female mice and oogenesis of premture female mice. Abd El-Rehim and Abu Amra (1992) reported also, that this extracted venom factor activates the process of spermatogensis of mature male mice .

This work was conducted in order to identify the possible effect *(in vivo)* of this factor on oocyte growth response of adult female fish; *Gambusia affinis* holbrookii at its resting stage during the winter. The process of spermatogenesis of adult male fish during the same season will be also investigated.

MATERIALS AND METHODS

Fourty adult male and female fish of *Gambusia affinis* Holbrookii were collected from the north eastern region of the River Nile at Sohag City through the winter which is considered after Nawar and Wahba (1966) as the resting stage of the

female. The fish were kept in well areated tanks filled with Nile water and continuously areated using air pumps. The fish were fed on fresh liver tissue of frogs.

Females were divided into two groups, the first group was injected i.p. with 0.1 ml saline solution each and regarded as control. The second group was injected with the same volume of a solution containing an extract that was previously isolated and purified from the venom of the Egyptian scorpion; *Buthus occitanus* to maintain a final dose 1 ug/g of body weight according to procedure described by Ferreira (1965). This dose was determined based on the LD_{50} of the venom extraction *(MEIER and THEAKSTON, 1986)*.

Ovaries were taken 24 hours after injection for fixation and after dehydration and embedding, sections were stained with haematoxylin and eosin (DRURY AND WALLINGTON, 1980).

The males were also divided into two groups, the first group acted as a control and each fish was injected i.p. with 0.1 saline solution. The second group was injected with 1 ug/g of body weight of the extracted factor similar to the female group. Testes were taken 24 hours after injection for routine histological studies as that previously described with female group.

RESULTS

I - Effect of the venom extract on the ovary :

The results in table (1) indicated that the injection of the isolated extract from the venom of the Egyptian scorpion; *Buthus occitanus* caused a complete absence of the degenerating ovocytes in the ovaries of treated fish (Fig. 2), which existed in the control one (Fig. 1).

Table (1) shows that the young ovocytes which are situated near the center of the ovary contact with the ovigorous fold in the treated ovaries (Fig. 2) were increased in number and size as compared to the non treated group (Fig. 1).

The mature ovocytes which are polygonal in shape and are charged with yolk granules were identified in ovaries of the treated fish only (table 1 & Fig. 2).

II- The effect of the venom extract on the testis :

The results presented in table (2) show that treating mature male gambusia with 1 ug/g b w of the extracted factor caused non appreciable changes in the number of primary, secondary spermatocytes and spermatids. The number of the immature spermatophores (Fig. 3 & 4) was decreased due to the

venom extraction treatment, but the number of mature spermatophores (Table 2) were increased significantly ($P<0.005$ Presented data are the mean number of ovocytes counted in 10 sections for each fish of control and treated ones.

The value of the young ovocytes represents the mean ± S.E and it is significant value.

Table (1): Effect of a single dose (1µg/gm body weight) of venom fraction isolated from *Buthus occitanus* on the number of ovocytes of *Gambusia affinis*.

Degenerating oocytes		Young ovocytes		Mature oocytes	
Control	Treated	Control	Treate	Control	Treated
27	---	11.6 ± 0.57	17.6 ± 0.755	—	15

Table (2): Effect of 1 µg/gm body weight of an extract isolated from the venom of the Egyptian scorpion *Buthus occitanus* on the spermatogenesis of mature male fish *Gambusia affinis*. Each result represents the mean count of each cell type in 50 section of 10 fish.

Ampullae		Mean S.E.
Primary spermatocytes	Control	3.28 ± 0.695
	Treated	3.42 ± 0.776
Secondary spermatocytes	Control	3.14 ± 0.496
	Treated	4.28 ± 0.610
Spermatids	Control	4.20 ± 0.521
	Treated	5.20 ± 0.464
Immature spermatophores	Control	7.4 ± 0.636
	Treated	4.4 ± 0.471
Mature spermatophores	Control	11.8 ± 0.783
	Treated	17.2 ± 1.041

($P<0.005$)

DISCUSSION

Experiments in the present study demonstrated that the injection of a sublethal dose isolated factor from the venoms of the Egyptian scorpion; *Buthus occitanus* causes a complete absence of degenerating ovocytes in the ovaries of treated fish the usual sign, of resting aovarian stage. This indicates that the extract initiated ovarian development. The young ovocytes were increased in number and size. Oocyte maturation is mainly characterized by the prominent increase of the number and size of the young ovocyte. Similar findings were described by Nawar and Wahba (1966). Interpretation of such results was based on the fact that the isolated venom could lead to enhance the oocyte maturation of the fish. An interesting finding was the appearance of mature ovocytes in the treated fish while in control fish there were no mature ovocytes which is the usual case with the female fish in the resting stage. It is suggested that the endogenous bradykinin that was probaly activated by the action of the venom extract enhanced the release of gonadotropin regulatory hormone. (LH-RH/FSH-RH), resulting in the elevation of leutinizing hormone (LH) and follice stimulating hormone (FSH) (Li, 1972), the hormones expected to promote ovarian growth.

It is accepted also that bradykinin can induce prostaglandin release in a variety of animal tissues (Croker and Willavays, 1976; Jeffery and Kinsella, 1983). However, one can suggest that the administered venom fraction might enhance the endogenous bradykinin leading to prostaglandin release and promoted the observed effect on ovarian growth. A recent contribution from our laboratory claimed that this venom extract enhances ovarian growth in premature female mice *(Nassar et al., 1990).*

The present results demonstrated the effectiveness of the venom fraction in stimulating the spermatogenesis in male gambusia. Although, the number of primary spermatocytes, secondary spermatocytes and spermatids showed insignificant change, the number of immature spermatophores was decreased significantly due to the venom extraction treatment. The number of mature spermatophores were increased significant ($P< 0.005$). It is clear that the increasing of the number of mature spermatophores was via to decreasing the number of immature spermatophores. Thus the isolated venom extraction could be considered effective in testis cellular differentiation leading to promotion of spermatogenesis in male gambusia. This promotion could be attributed to the activation of the endogenous bradykinin by the action of this activating injected venom fraction. Such an activated bradykinin may turn enhance

growth factor(s), that regulates cellular growth. *SCHILL et al., (1982)* considered that kinins to be primary responsible for spermatogenesis. Moreover, kallikrein has been reported to increase the relative number of spermatocytes and first appearance of A-spermatogonia (1-2 days ealier) in premature rats *(ROHEN and STUTTMANN, 1975)*, to increase the incorporation of 3H thymidine into DNA of the testicular tissue of rats *(MATHIESSEN and ROHEN, 1975)*, and to enhance glucose intake *(BLUMEL et al., 1975)*. *YOSHIYUKI et al., (1991)* reported that prostaglandin F_{2a} stimulates proliferation of clonal osteoblastic MC3 T3-El cells. Abd-El Rehim and Abu Amra, (1992) reported that this isolated bradykinin potentiating activity of the venom of *Buthus occitanus* enhances spermatogenesis in premature and mature mice respectively.

REFERENCES

Abd El-Rehim, S.A. (1990): Physiological studies of a separated fraction from the venom of an Egyptian scorpion on the living cells of some organs. Ph. D. Thesis, Assiut Univ. (Sohag).

Abd El-Rehim, S.A. and *Abu Amra, S. (1992):* Effect of isolated venom fraction of the Egyptian scorpion, *Buthus occitanus* on the count of intestinal cells of testes and testosterone level. J. Egypt. Ger. Soc. Zool., 9(A): 91-103.

Blumel, G.; Erhardt, W.; Rupp, N. and Huber, P. (1975): Microangio-graphic investigation following kallikrein application. In: Harberland, G.L.; Rohen, J.W.; Schirren, C. and Huber, P. (eds.). Kininogenases. Kallikrein 2. Schattauer, stuttgart New York. pp. 107-108.

Cherry, P.D.; Furchgott, R. F.; Zawadzki, T.V. and Dan, D. (1982): Role of endothelial cells in relaxation of isolated arteries by bradykinin. Proc. Natl. Sci. U.S.A., 79: 2105-2110.

Croker, A.D., and Willavays, S.P. (1976): Possible involvement of Prostaglandins in the contractile action of bradykinin on rat terminal ileum. J. Pharm. Pharmac., 28: 78-79.

Drury, R.A.B., and Wallington (1980): In Carleton's Histological technique, 5th. ed. pp. 36-102. Oxford Univ. Press.

Espey, L.L. (1980): Ovulation as an inflammatory reaction-hypothesis. Biol. Repord. 23: p. 37.

Espey, L.L.; Miller, D.H. and Margoluis, H.S. (1986): Ovarian increase in Kinin-generating cepacity in the PHSc/ H C G-primed immature rat. Am. J. Physiol., 251: p. 362.

Ferrira, S. H. (1965): A bradykinin potentiating factor (BPF) present in the venom of *Buthrops jararaca.* Br. H. Pharmacol., 24: 163-164.

Goldstein, R. H. and Wall, M. (1984): Bradykinin stimulation of phosphoinositide hydrolysis in guinea-pig ileum longitudinal muscle. Br. J. Pharmacol., 105: 919-924.

Jeffery, Poul and Kinsella, J. E. (1983): Effects of bradykinin and bovine serum albumin on arachidonic acid and prostaglandins release from perfused rat heart. Prostaglandins leukotrienses and medicine, II: 419 - 430.

Kato, H. and Suzuki, T. (1970): Structure of bradykinin potentiating peptide containing tryptophan from the venom of *Agkistrodon halys bolombffii.* Experimentia, 26: p. 1205.

Kleeberg, S.; Pronzen, R. and Leidl, W. (1975): Effect of kallikrein on the gonads of premature male rats. In: Harberland, G. L.; Rohen, J. W. Schirren C. and Huber, P. Kininogenases-Kallikrein 2. Schattauer, Stuttgart. New York, pp. 99-105.

Li, C. H. (1972): Hormones of adenohypothyesis Proc. Am. Phil. Soc., 116: 365-328.

Marceau, F. and Tremblay, B. (1986): Des-Arg9 bradykinin modulatES DNA synthesis, phospholipase C, and protein kinase C in cultured mesangial cells. Life Sci., 39: 2351-2358.

Mathiessen, P. F. and Rohen, J. W.; (1975): ^{3}H-thymidine-incorporation in the testis of the albino rat after migration of the pancreatic duct. In: Harberland, G.L.; Rohen, J. W.; Schirren, C. and Huber, P. (eds). Kininogenases. Kallikerin 2. Schattauer, Stuttgart. New York, pp. 75-83.

Meier, J. and Theakston, R. D. G. (1986): Approximate LD_{50} determination of snake venoms using eight to ten experimental animals. Toxicon, 24 (4): 395-401.

Mohamed, A. H. and Khaled, L. Z. (1969): Effect of *Cerastes cerastes* venom on blood and tissue histamine and on arterial blood pressure. Toxicon, 6: 221-223.

Nassar, A. Y.; Abu-Sinna, G. and Abdel-Rahim, S. (1990): Effect of bradykinin potentiating fraction from venom of Egyptian scorpion, *Buthus occitanus,* on the ovaries and endometrium of mice. Toxicon, 28: 525-534.

Nassar, A. Y.; Abu-Sinna, G. and Abu-Amra, S. (1989): Isolated fractions from toxins of Egyptian scrpions and cobra, activated smooth muscle contraction and glomerular filtration. Toxicon, 27: p. 57.

Nawar, G. and Wahba, M. T. (1966): The gonads and the breeding habits of the cyprinodont *Gambusia affinis holorookii.* Bull. Sci. and Tech., 9: 67-82.

Oshima, G.; Satoomori, T. and Suzuki, T. (1969): Distribution of proteinase, argenin ester hydrolas and kinin releasing enzymes in various kinds of snake venom. Toxicon, 7: p. 229.

Rohen, J. W. and Buschuter, H. (1975): Karyometric measurements on the Sertoli cell nuclei in kallikrein-treared albino rats. In: Huber, p. (eds.) kininogenases. kallikrein 2. Schattauer, Stuttgart. New York, pp. 85-97.

Rohen, J. W. and Stuttmann, R. (1975): The early postnatal development of the germinative epithelium of the testis in the albino rat under the influence of kallikrein. In: Harberland, G. L.; Rohen, J. W. and Suzuki, T. (eds.) kininogenases. kallikrein 4. Schattauer, Stuttagrat. New York, pp. 217-223.

Schill, W. B.; Bain, J. and Schwarzstein, L. (1982): Treatment of male infertility. Springer-Verlag Berlin Heidelberh and New York. (1st ed.).

Smith, C. and Perks, A. M. (1983): The kinin system and ovulation: changes in plasma kininogenases and in kinin-forming enzymes in the ovaries and blood of rats with 4-days estrous cycles. Can. J. Physiol. Pharmacol., 61: p. 736.

Suzuki, T.; Iwanage, S.; Sato, T.; Nagazawa, S.; Kato, H.; Yono, M. and Heriuchi, K. (1967): In international symposium on Vaso-active polypeptides: Bradykinin and related kinins Rochae Silva, M. and Rothschild, H. A., ed., Sao Paulo, Brazil, Edart Livraria Editora Lt. da., P. 27.

Yoshimura, Y.; Espey, Y.; Hosoi, T.; Adachi, S.S.; Atlas, R. B.; Ghodgaonkar, N. and Wallach, E. E. (1988): The effect of bradykinin on ovulation prostaglandin production by the perfused rabbit ovary. Endocrinology, 122: 2540-2546.

Yoshiyuki, H.; Shun-ichi, H.; Toshio, M.; Ken-ichi, T.; Kiyoshi, H.; Hiroaki, K.; Tamotsu, H.; Etsuro, O. and Masayoshi, K. (1971): Prostaglandin F_{2a} stimulates proliferation of clonal osteoblastic MC3 T3-E1 cells by up regulation of Insulin-like growth factor I receptors. J. Biol. Chem., 266 (31): 21044-21050.

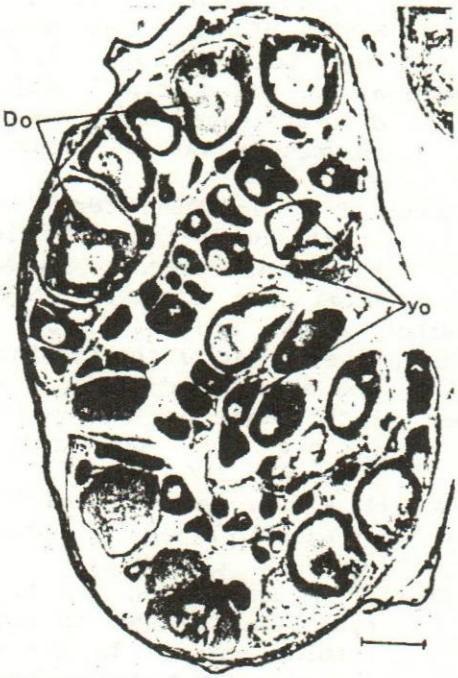

Fig 1 : A photomicrogragh of a cross section of the oyary of control Fish Gambusia. It shows normal number of degenerating and young ovocyts. H. and E. (X 100)
D.O = Degenerating ovocyte.
Y.O = Young ovocyte.
Scale bar = 0.01 mm.

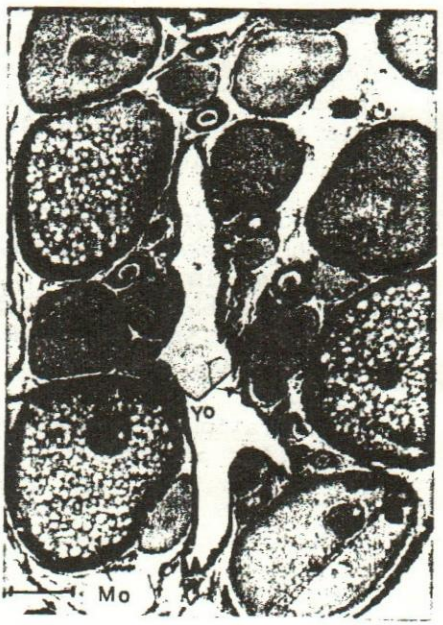

Fig 2 : A photomicrograph of a cross section of ovary of Gambusia fish treated with 1/ug of extracted factor / gm bodg weight. It shows a completely absence of degenerating ovocytes, an increase in the number and size of the young ovocytes as well as existance of the mature ovocytes. H. and E. (X 100)
Y.O = Young ovocyte .
M.O = Mature ovocyte.
Y.G = Yolk granules.
Scale bar = 0.01 mm.

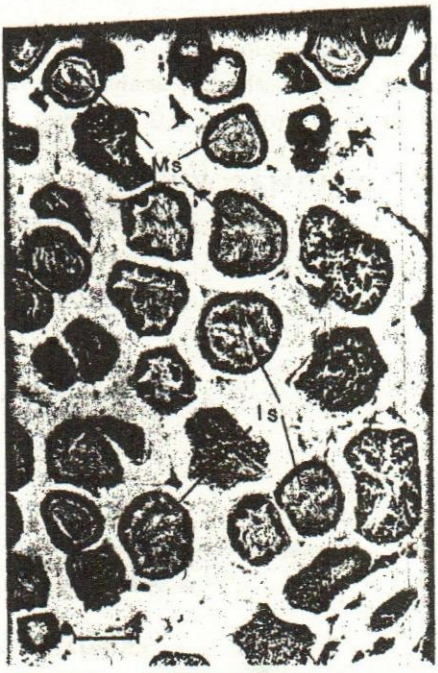

Fig 3 : A photomicrograph of a cross section of testis of a control Gambusia fish. It shows a normal number of immature and mature spermatophores. H. and E. (X 200)
I. S = Immature spermatophores.
M. S = Mature spermatophores.
Scale bar = 0.01 mm.

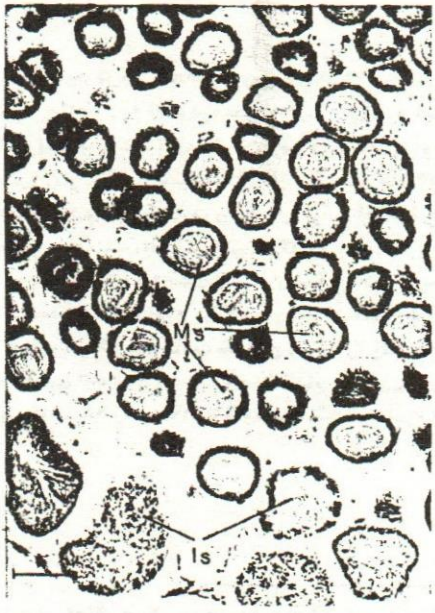

Fig 4 : A photomicrograph of a cross section of testis of Gambusia treated with 1/ ug of extracted factor/gm body weight. It shows a decrease in the number of immature spermatophores and an increase in the number of the mature spermatophores. H. and E.(X 200)
I. S = Immature spermatophores
M. S = Mature spermatophores.
Scale bar = 0.01 mm.