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DEVELOPMENT OF THE POSTERIOR SEROUS LINGUAL GLANDS (VON EBNER'S GLANDS) IN THE RABBIT (*ORYCTOLAGUS CUNICULUS*)

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Abstract. The first signs of formation of the posterior serous lingual glands (von Ebner's glands) were observed at day 22 of prenatal development of the rabbit. The development of glands was initiated by the appearance of epithelial bands, growing into the connective tissue. At day 26 p.c. the epithelial bands were longer. At day 1 of postnatal life the secretory parts of glands were formed together with excretory parts. Carbohydrates were found in the lumen of the excretory parts. At day 15 p.p. it was shown that the excretory parts opened at the bottom of furrows of foliate and circumvallate papillae. In the successive periods of life in the rabbit were observed linear arrangement for excretory parts opening at the bottom of furrows of foliate papillae. Excretory parts which opened at the bottom of circumvallate papillae formed a circular system.

Key words: rabbit, tongue, development, serous glands

INTRODUCTION

The oral cavity is a segment of the alimentary tract, in which the process of digestion is initiated. An important role played by this section is also to perform mechanical processing and wetting of food in order to form food morsels, thanks to which it may be easily passed to farther sections of the alimentary tract. The secretion of small and large salivary glands is used to moisten food. The former include lingual, labial, buccal, molar and palatine glands. The lingual glands in mammals are seromucous anterior lingual glands (Nühn's glands) located in the tip of the tongue, posterior serous lingual glands (von Ebner's glands) and posterior mucous lingual glands (Weber's glands), [Zawistowski 1986, Nagato et al. 1997, Zabel 2000]. We may find information in available literature on the structure of both small lingual glands [Sanford and Josephson 1989, Azzali et al. 1989, Gargiulo et al. 1995, Nagato et al. 1997] and large salivary glands [Toyoshima and Tandler 1991, Marin et al. 1992] in the human and animals. However,

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there is little information on developmental morphology of small salivary glands [Fujimoto et al. 1993]. Results of investigations presented by the author concern mainly the formation of large salivary glands [Merida-Velasco et al. 1991, Hayakawa et al. 1992, Umeda et al. 2001, Tucker 2007].

The present paper describes the morphogenesis of posterior serous lingual glands (von Ebner's glands) from day 15 of prenatal development to 6th month of postnatal life in the rabbit, popular laboratory animal [Červený and Míšek 1993, Weisbroth et al. 1974].

MATERIAL AND METHODS

Studies were conducted on 123 tongues of the rabbits (*Oryctolagus cuniculus f. domestica*), of both sexes, being at day 15, 18, 20, 22 and 26 of prenatal life (p.c.) and from rabbits at day 1, 15 and 30 and in the 6th month of postnatal life (p.p.). The age of fetuses was determined on the basis of the CRL value, [Evans and Sack 1993].

Materials for obserwations under a light microscope (LM) were fixed in 10% neutralized formalin and Bouin's solution. Fixed specimens of the tongues were dehydrated in alcohols with increasing concentrations (50–96%), embedded in paraplast and sliced into sections with thickness ranging from 3 to 5 μm in the median, transverse and dorsal planes. The Masson-Goldner staining, HE and PAS were applied in this study.

Materials for observations under a scanning electron microscope (SEM) were fixed in the Karnovski solution. For the purpose of observations of the connective tissue core the samples after being fixed were placed in a 10% NaOH solution and dehydrated in alcohols. The specimens were mounted on aluminium stubs covered with carbon tabs and sputtered with gold. Observations were made with a Hitachi S-4200.

RESULTS

In the period between day 15 and day 20 of prenatal development in the rabbit no signs of formation of posterior serous lingual glands were found.

At day 22 p.c. on the site of two primordia of circumvallate papillae circular epithelial bands were shown, which they grew into the lamina propria of the lingual mucosa (Fig. 1). Apart from that, on the dorsolateral side of the posterior section of the body of the tongue, on the site of the primordia of foliate papillae parallel epithelial bands were observed (Fig. 2). Numerous mitoses were observed in the epithelial bands. Epithelial bands were symptoms of the onset of development of posterior serous lingual glands.

In the successive, 26th day of prenatal development epithelial bands of primordia of circumvallate and foliate papillae were in some places longer and grew deep into the lamina propria of the mucosa, reaching the lingual muscles (Figs. 3 and 4). After removal of epithelial cells using a 10% NaOH solution depressions were shown in sites, in which epithelial bands grew in deeper (Fig. 5).

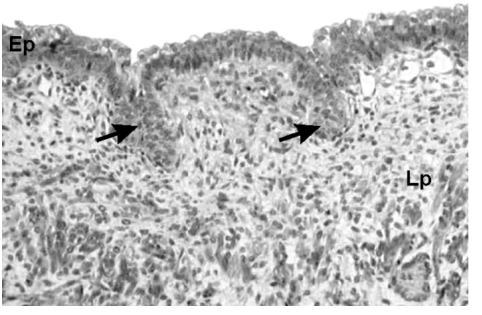

Fig. 1. Transverse section of the primordium of circumvallate papilla at day 22 p.c., Ep – epithelium, Lp – lamina propria of the mucosae, arrows indicate circular epithelium band, LM, x 12.5

Ryc. 1. Przekrój poprzeczny zawiązka brodawki okolonej w 22. dniu p.c., Ep – nabłonek, Lp – blaszka właściwa błony śluzowej, strzałki wskazują koliste pasmo nabłonkowe, LM, x 12.5

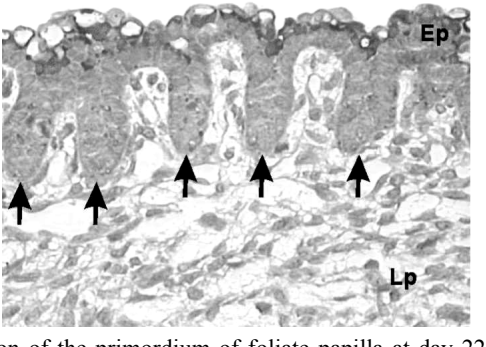

Fig. 2. Transverse section of the primordium of foliate papilla at day 22 p.c., Ep – epithelium, Lp – lamina propria of the mucosae, arrows indicate parallel epithelium bands, LM, x 25

Ryc. 2. Przekrój poprzeczny zawiązka brodawki liściastej w 22. dniu p.c., Ep – nabłonek, Lp – blaszka właściwa błony śluzowej, strzałki wskazują równoległe pasma nabłonkowe, LM, x 25

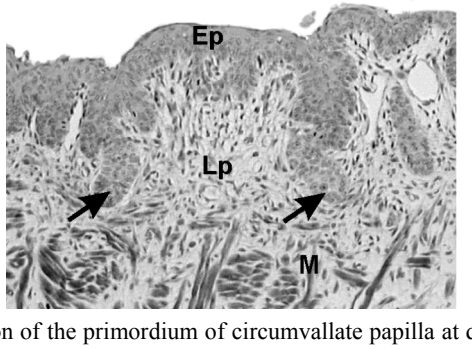

Fig. 3. Transverse section of the primordium of circumvallate papilla at day 26 p.c., Ep – epithelium, Lp – lamina propria of the mucosae, M – muscles of the tongue, arrows indicate longer epithelium bands, LM, x 12.5

Ryc. 3. Przekrój poprzeczny zawiązka brodawki okolonej w 26. dniu p.c., Ep – nabłonek, Lp – blaszka właściwa bony śluzowej, M – mięśnie języka, strzałki wskazują dłuższe pasma nabłonkowe, LM, x 12,5

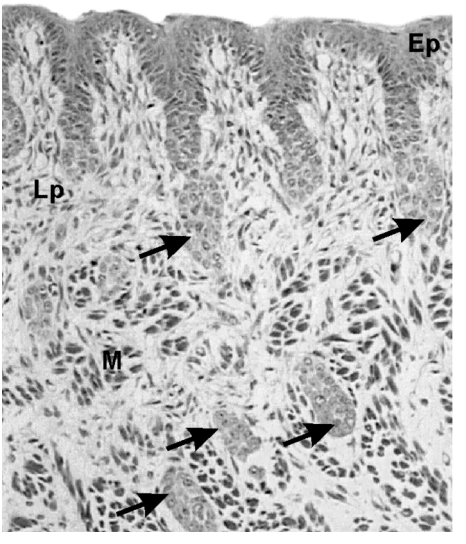

Fig. 4. Transverse section of the primordium of foliate papilla at day 26 p.c., Ep – epithelium, Lp – lamina propria of the mucosae, M – muscles of the tongue, arrows indicate longer epithelium bands, LM, x 12.5

Ryc. 4. Przekrój poprzeczny zawiązka brodawki liściastej w 26. dniu p.c., Ep – nabłonek, Lp – blaszka właściwa błony śluzowej, M – mięśnie języka, strzałki wskazują dłuższe pasma nabłonkowe, LM, x 12,5

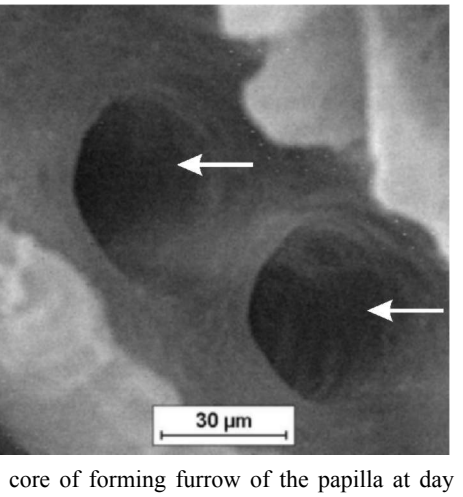

Fig. 5. Connective tissue core of forming furrow of the papilla at day 26 p.c., arrows indicate sites, in which epithelial bands grew in deeper (Fig. 5)

Ryc. 5. Zrąb łącznotkankowy formującego się rowka brodawki w 26. dniu p.c., strzałki wskazują miejsca, w których pasma nabłonkowe wrastały głębiej

At day 1 p.p. in the rabbit farther sections of epithelial bands bifurcated and they were transformed into secretory parts of glands. At that time their lumen was not visible. Epithelial bands split, in this way forming excretory parts of glands. Walls of the excretory parts were formed from two layers of cells. These ducts were not found to have contact with the surface of the tongue (Fig. 6). PAS staining showed a positive result indicating the presence of carbohydrates in the lumen of developing excretory parts of posterior serous lingual glands.

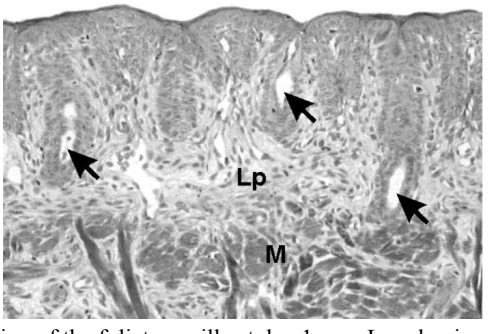

Fig. 6. Transverse section of the foliate papilla at day 1 p.p., Lp – lamina propria of the mucosae, M – muscles of the tongue, arrows indicate excretory parts of the posterior serous lingual glands, LM, x 6.3

Ryc. 6. Przekrój poprzeczny brodawki liściastej w 1. dniu p.p., Lp – blaszka właściwa błony śluzowej, M – mięśnie języka, strzałki wskazują przewody wyprowadzające gruczołów językowych tylnych surowiczych, LM, x 6,3

Based on microscopic observations conducted at day 15 p.p. it was shown that primary epithelial bands split over the entire length, forming excretory parts of glands. At the farther ends the excretory parts of glands bifurcated and formed intercalated ducts ending with serous secretory parts. The lumen of follicles was visible (Fig. 7). Myoepithelial cells were observed in the vicinity of pyramidal serous cells forming secretory follicles. Secretory parts and excretory parts of posterior serous lingual glands were observed both in the area of the lamina propria of the mucosa and deep among lingual muscles. Secretory parts formed racemoses. The presence of carbohydrates was shown in the lumen of excretory parts and intercalated ducts.

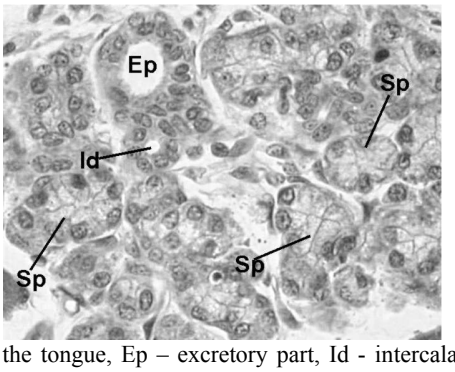

Fig. 7. Dorsal section of the tongue, Ep – excretory part, Id - intercalated duct, Sp – secretory parts, LM, x 40

Ryc. 7. Przekrój grzbietowy języka, Ep – przewód wyprowadzający, Id – wstawka, Sp – odcinki wydzielnicze, LM, x 40

At day 30 p.p. and in the 6th month p.p. earlier observations on the distribution and structure of posterior serous lingual glands were confirmed on the basis of microscopic observations. Excretory parts of glands opened at the bottom of furrows of circumvallate and foliate papillae. Occasionally their openings were found on the surface of circumvallate papillae. Below circumvallate papillae excretory parts were arranged in circles (Fig. 8), while below foliate papillae they were arranged linearly (Fig. 9). The arrangement of these ducts was consistent with the course of furrows of gustatory papillae.

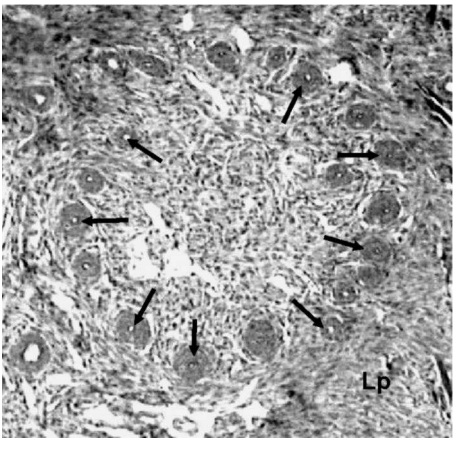

Fig. 8. Dorsal section of the tongue on the site of circumvallate papilla, arrows indicate excretory parts arranged in circles, Lp – lamina propria of the mucosae, LM, x 6.3

Ryc. 8. Przekrój grzbietowy języka w miejscu brodawki okolonej, strzałki wskazują przewody wyprowadzające ułożone koliście, Lp – blaszka właściwa błony śluzowej języka, LM, x 6.3

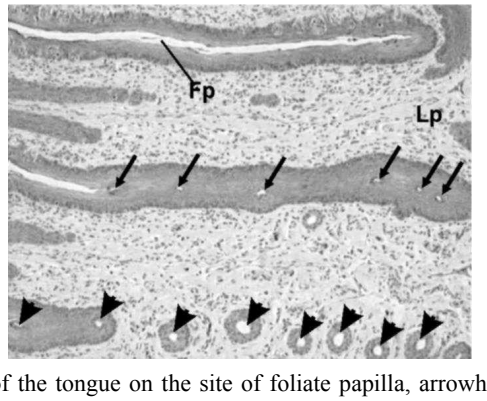

Fig. 9. Dorsal section of the tongue on the site of foliate papilla, arrowheads indicate excretory parts arranged linearly, arrows – excretory parts opening at the bottom of furrows of papilla, Fp – furrow of foliate papilla, Lp – lamina propria of the mucosae, LM, x 6.3

Ryc. 9. Przekrój grzbietowy brodawki liściastej, groty wskazują przewody wyprowadzające ułożone liniowo, strzałki – przewody wyprowadzające otwierające się na dnie rowka brodawki, Fp – rowek brodawki liściastej, Lp – blaszka właściwa błony śluzowej, LM, x 6.3

DISCUSSION

Excretory parts of small and large salivary glands open to the oral cavity. Large salivary glands contribute to the production of approx. 90% saliva [Tucker 2007]. The other 10% saliva is produced by small salivary glands. Different types of salivary glands produce a type of saliva differing in terms of its chemical composition. The composition of saliva, or rather its mixture formed as a result of secretions of different salivary glands being mixed together, is important in the process of digestion of food typical of a given animal species and the human. The initiation of digestion in the oral cavity requires the presence of digestive enzymes, which concentration is connected with the fact whether the species is carnivorous, herbivorous or omnivorous.

According to Matsuo [2000], saliva may affect the sensitivity of taste perception. It also plays an important role in the transport of taste substances to receptors and their protection.

The distribution of the posterior serous lingual glands corresponded with those previously described [Hand 1970, Riva et al. 1999].

All glands are of epithelial origin. Thus a mechanism of their development is similar. Literature describes the development of mucous glands based on large salivary glands in mammals [Hayakawa et al. 1992, Tucker 2007]. The first signs of formation of salivary glands, which were described on the basis of microscopic observations, were epithelial thickenings, which protrude into the underlying mesenchyme. A primordium of the gland was formed from the thickening, linking the developing gland with the oral cavity. In the site of the primordium elongating in the connective tissue the primary excretory part is formed [Fujimoto et al. 1993, Tucker 2007]. Similar observations were recorded in the studies on the development of posterior serous lingual glands in the rabbit. The development of large salivary glands, primarily the excretory part, which was presented in a study by Garcia-Garcia et al. [1991], was also preceded by changes within the epithelium.

Elongation of epithelial bands was determined by numerous mitoses, while the formation of the duct lumen was subjected to processes of apoptosis [Tucker 2007].

Treatment of tissues with 10% NaOH solution facilitated observations concerning the structure of the connective tissue core in sites, where efferent ducts of glands opened. Similar observations showing furrows of foliate papillae, at the bottom of which glands open, were presented by Kobayashi [1992].

Formation of salivary glands is a complex process occurring during both pre- and postnatal life Tucker [2007].

Morphogenesis of salivary glands, similarly as other organs, is a process in which epithelial-mesenchymal interaction occurs [Lawson 1972, Mackenzie 1984].

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ROZWÓJ GRUCZOŁÓW JĘZYKOWYCH TYLNYCH SUROWICZYCH (GRUCZOŁÓW VON EBNERSA) U KRÓLIKÓW (*ORYCTOLAGUS CUNICULUS*)

Streszczenie. Pierwsze oznaki formowania się gruczołów językowych tylnych surowiczych zaobserwowano w 22. dniu rozwoju prenatalnego królika. Rozwój gruczołów zapoczątkowało pojawienie się pasm nabłonkowych, wrastających na obszar tkanki łącznej. W 26. dniu p.c. pasma nabłonkowe były dłuższe. W 1. dniu życia postnatalnego z pasm nabłonkowych formowały się odcinki wydzielnicze gruczołów oraz przewody wyprowadzające. W świetle przewodów wyprowadzających stwierdzono obecność cukrowców. W 15. dniu p.p. wykazano, że przewody wyprowadzające uchodziły na dnie rowków brodawek liściastych i okolonych. W kolejnych badanych okresach życia królika zaobserwowano liniowy układ przewodów wyprowadzających uchodzących na dnie rowków brodawek liściastych. Przewody wyprowadzające, które otwierały się na dnie rowków brodawek okolonych, tworzyły układ kolisty.

Słowa kluczowe: królik, język, rozwój, gruczoły surowicze

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DEVELOPMENT OF THE TONGUE IN THE RABBIT (*ORYCTOLAGUS CUNICULUS F. DOMESTICA*) AND THE ORDER OF FORMATION OF LINGUAL PAPILLAE IN PRE- AND POSTNATAL LIFE

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Abstract. A total of 129 tongues were analyzed, which were collected post mortem from rabbits *(Oryctolagus cuniculus f. domestica)*, aged from day 15 of prenatal development (p.c.) to the 6th month of postnatal life (p.p.). The tongue was formed on the basis of its primordia starting as early as day 15 p.c. At that time the first primordia of lingual papillae were observed, which were primordia of circumvallate papillae. At day 18 p.c. the presence of primordia of fungiform papillae was shown, while at day 22 p.c. primordia of foliate and filiform papillae were found. On the dorsal surface of the tongue during the development the torus and median sulcus of the tongue were formed. The median sulcus ended in front of the torus of the tongue. The tongue in the rabbit increased in length from day 22 p.c. to the 6th month p.p. from 9 mm to 65 mm. During that time the weight of the tongue increased from 0.05 g to 9.59 g.

The development of the final form of the tongue is a long-term process and occurs both during the prenatal and postnatal life. First primordia of gustatory papillae appear and only later primordia of mechanical papillae are formed.

Key words: tongue, development, papillae

INTRODUCTION

The manner of the formation of the tongue from its primordia during embryogeny has already been clarified. Numerous handbooks on embryology provide information on that subject [Bartel 2002, Rüsse and Sinowatz 1991, Sadler 1993, Zietzschmann and Krölling 1955]. However, there is insufficient data on the period of pregnancy, during which these changes take place, particularly in case of very numerous animal species. This is connected, among other things, with the diversified duration of pregnancy in different species. For this reason this subject is still being investigated in many studies.

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The rabbit is a popular laboratory animal, which anatomy [Krause 1884, Tierientiew et al. 1952] and physiology [Habermehl 1975, 1980, Kaplan and Timmons 1979] have already been thoroughly investigated and described. Numerous literature sources have focused on the rabbit due to the fact that it is also a breeding animal, which supplies meat, wool and leather [Cholewa 2000, Herman 1963]. However, there is insufficient information on developmental changes occurring during pre- and postnatal life in the rabbit when certain organs are formed, including also the tongue. A vast majority of studies concerning the tongue focus on the formation of lingual papillae and changes in their structure [Iwasaki et al. 1996, 1997, Tichý 1992, Witt and Reutter 1997]. Some publications describe results of studies concerning the development of taste buds, their ultrastructure and numbers [Miller and Chaudhry 1976, Robinson and Winkles 1990, Tichý 1994, Witt and Reutter 1996]. Little attention has been focused on the development of the tongue as a whole, taking into consideration its shape and metric characteristics. For this reason the aim of this study was to supplement knowledge on the development of the tongue and to emphasize the role of prenatal life, in which individual types of lingual papillae are formed.

MATERIAL AND METHODS

Studies were conducted on 129 tongues of the rabbit (*Oryctolagus cuniculus f. domestica*) of both sexes, being at day 15, 18, 20, 22 and 26 of prenatal life (p.c.) and from rabbits at day 1, 15 and 30 and in the 6th month of postna tal life (p.p.). The age of fetuses was determined on the basis of the CRL (Crown-Rump-Length), [Evans and Sack 1973].

Tongues of fetuses being in their day 22 and 26 p.c. as well as tongues of animals being in their day 1, 15 and 30 p.p. and the 6th month p.p. were dissected free from the oral cavity. Dissected tongues were weighed and next fixed in 10% neutralized formalin or in Bouin's solution. After fixation the length and width of individual parts of tongues, i.e. the apex, the body and the root of the tongue, were measured using a ruler. Whole heads were dissected from animals being in their day 15, 18 and 20 of prenatal development and next they were fixed. Photographic documentation of the tongues was prepared using a digital camera Sony DSC-S75.

In order to prepare histological specimens smaller samples were collected from the apex, the body and the root of the tongue, which were dehydrated after fixation in series of alcohols with increasing concentrations (50–96%), embedded in paraplast and sliced in three planes, i.e. median, transverse and dorsal, into series slices with the thickness of 3 to 5 μm. Whole heads were used for histological preparation in case of animals being in their day 15, 18 and 20 p.c. The following staining methods were applied in the study: Masson-Goldner and hematoxylin-eosin (HE). Microscopic observations were conducted using a Jenaval (Carl Zeiss Jena) microscope.

RESULTS

In the rabbit at day 15 of prenatal development the tongue was already formed and its anatomical parts could be distinguished, i.e. the apex, the body and the root of the tongue. In the period from day 15 to 20 p.c. the tongue was arched on the dorsal side (Fig. 1).

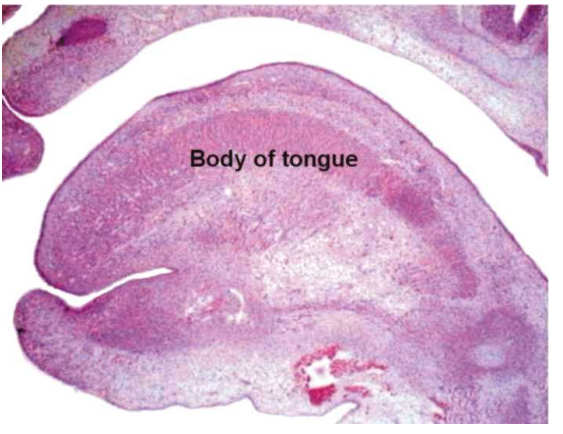


Fig. 1. Median section of the tongue at day 15 p.c. (LM, x 3, Masson-Goldner staining)
Ryc. 1. Przekrój strzałkowy języka w 15. dniu p.c. (LM, x 3, barwienie Masson-Goldner)

In the analyzed day 15 of prenatal development on the tongue in the rabbit the presence of the first primordia of lingual papillae was observed. The first which could be observed were two primordia of circumvallate papillae. They took the form of epithelial thickenings and were arranged on the root of the tongue symmetrically on both sides of the median plane of the tongue (Fig. 2).

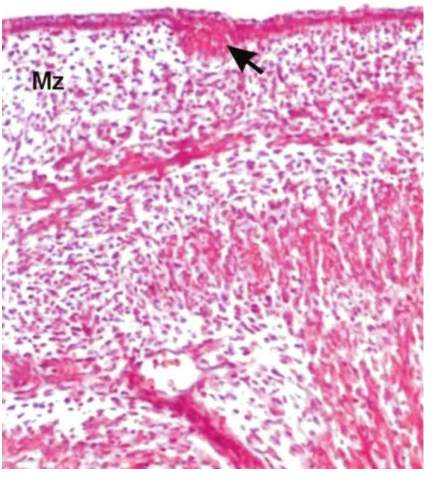


Fig. 2. Median section of the tongue: arrow indicates primordium of vallate papilla, Mz – mesenchyme (LM, x 12,5, HE staining)
Ryc. 2. Przekrój strzałkowy języka: strzałka wskazuje zawiązek brodawki okolonej, MZ – mezenchyma (LM, x 12,5, barwienie HE)

In the successive day of analysis, i.e. day 18 p.c. on the dorsal surface of the apex and its margins as well as the dorsal surface of the body of the tongue the presence of primordia of fungiform papillae was observed. They were found, similarly as in case of primordia of circumvallate papillae, in the form of epithelial thickenings (Fig. 3). Similar observations were recorded at day 20 p.c.

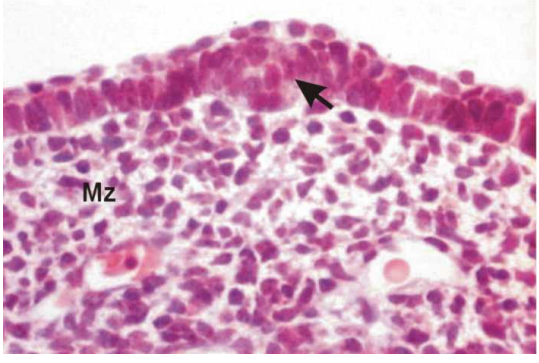
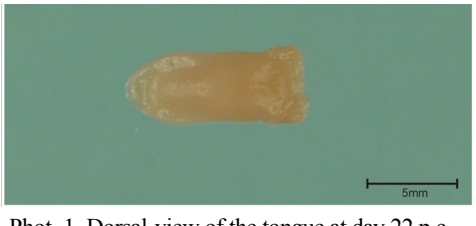
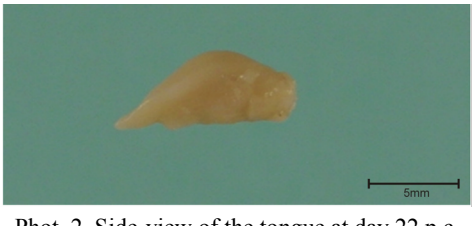


Fig. 3. Transverse section of the tongue: arrow – primordia of fungiform papilla, Mz – mesenchyme (LM, x 40, HE staining)
Ryc. 3. Przekrój poprzeczny języka: strzałka – zawiązek brodawki grzybowatej, Mz – mezenchyma (LM, x 40, barwienie HE)

At day 22 of fetal development the tongue of rabbits was elongated and arched dorsally. The apex of the tongue was flattened and its end narrowed (Phot. 1 and 2).



Phot. 1. Dorsal-view of the tongue at day 22 p.c.
Fot. 1. Widok grzbietowy języka w 22. dniu p.c.



Phot. 2. Side-view of the tongue at day 22 p.c.
Fot. 2. Widok boczny języka w 22. dniu p.c.

On the apex and the root of the tongue numerical values describing its width were bigger that those which describe its length. The length of the lingual body was bigger than its width. The mean total length of the tongue was 9 mm. The longest part of the tongue was the body of the tongue (Tab. 1). The mean weight of the tongue was 0.05 g (Tab. 2).

At day 22 p.c. microscopic observations confirmed further differentiation of the lingual mucosa in the rabbit. On the tongue the presence of two primordia of foliate papillae and numerous primordia of filiform papillae was observed. Primordia of foliate papillae were found on the posterior part of the body of the tongue, at the transition of the dorsal surface of the lingual body into its lateral surfaces. The appearance of primordia of foliate papillae was heralded by primary epithelial bands growing into the connective tissue (Fig. 4).

Table 1. Length and width of the tongue of the rabbits aged from day 22 p.c. to month 6 p.p. (mm)
Tabela 1. Długość i szerokość języka królika w wieku od 22. dnia p.c. do 6. miesiąca p.p. (mm)

Age Wiek	Parts of tongue Części języka					
	Apex of tongue Wierzchołek języka		Body of tongue Trzon języka		Root of tongue Korzeń języka	
	Length Długość	Width Szerokość	Length Długość	Width Szerokość	Length Długość	Width Szerokość
Day 22 p.c. 22. dzień p.c.	2	3,5	5	4	2	2,5
Day 26 p.c. 26. dzień p.c.	3	5	8	6	3	3,5
Day 1 p.p. 1. dzień p.p.	4	7	12	8	4	6
Day 15 p.p. 15. dzień p.p.	7	10	16	11	6	7
Day 30 p.p. 30. dzień p.p.	12	11	27	13	7	10
Month 6 p.p. 6. miesiąc p.p.	16	15	37	17	12	15

Table 2. Weight of the tongue of the rabbits aged from day 22 p.c. to 6 p.p. (g)
Tabela 2. Masa języka królika w wieku od 22. dnia p.c. do 6. miesiąca p.p. (g)

Age – Wiek	Weight – Waga
Day 22 p.c. – 22. dzień p.c.	0,05
Day 26 p.c. – 26. dzień p.c.	0,22
Day 1 p.p. – 1. dzień p.p.	0,52
Day 15 p.p. – 15. dzień p.p.	1,63
Day 30 p.p. – 30. dzień p.p.	3,97
Monath 6 p.p. – 6. miesiąc p.p.	9,59

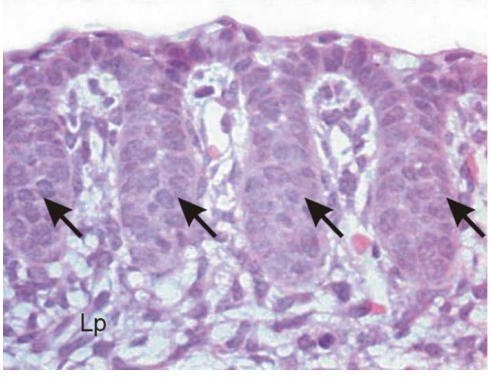


Fig. 4. Transverse section of the primordia of the foliate papilla at day 22 p.c.: arrows – primary epithelial streaks, Lp – lamina proporia of the mucosae (LM, x 40, HE staining)
Ryc. 4. Przekrój poprzeczny zawiązka brodawki liściastej w 22. dniu p.p.: strzałki – pierwszorzędowe pasma nabłonkowe, Lp – blaszka właściwa błony śluzowej (LM, x 40, barwienie HE)

Primordia of filiform papillae were distributed over the entire dorsal surface of the apex, the body and the margins of the tongue (Fig. 5).

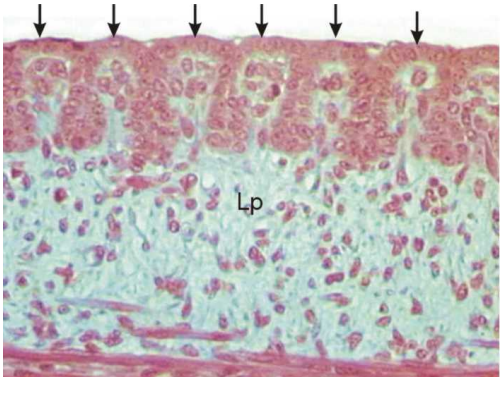
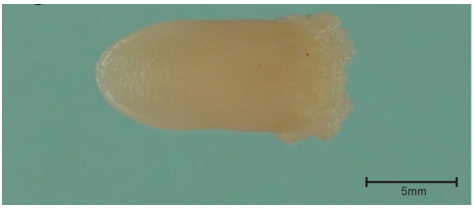


Fig. 5. Transverse section of the tongue at day 22 p.c.: arrows indicate primordium of filiform papillae, Lp – lamina proporia of the mucosae (LM, x 25, Masson-Goldner staining)
Ryc. 5. Przekrój poprzeczny języka w 22. dniu p.c.: strzałki wskazują zawiązki brodawek nitkowatych, Lp – blaszka właściwa błony śluzowej (LM, x 25, barwienie Masson-Goldner)

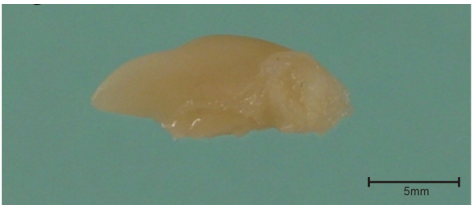
At day 22 of prenatal development in the rabbit on the tongue there were already all the types of primordia of both gustatory and mechanical papillae.

At the successive 26th day of intrauterine life in the rabbit, as a result of development in the visceral cranium and thus the development of the oral cavity, mean values describing length and width of the apex, the body and the root of the tongue were observed to increase. The width of the apex and the root of the tongue was still bigger than the length of these parts of the tongue. The width of the body of the tongue was smaller than its length (Tab. 1).

At day 26 p.c. the lingual apex was rounded. Margins of the tongue were tilted dorsally. On the body of the tongue an outline of the forming torus of the tongue could be observed (Phot. 3 and 4). The mean weight of the tongue increased by 0.17 g (Tab. 2).

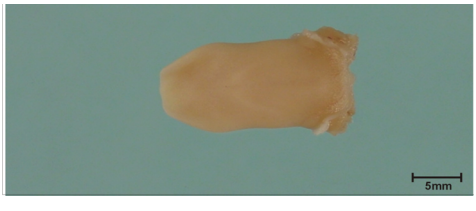


Phot. 3. Dorsal-view of the tongue at day 26 p.c.
Fot. 3. Widok grzbietowy języka w 26. dniu p.c.



Phot. 4. Side-view of the tongue at day 26 p.c.
Fot. 4. Widok boczny języka w 26. dniu p.c.

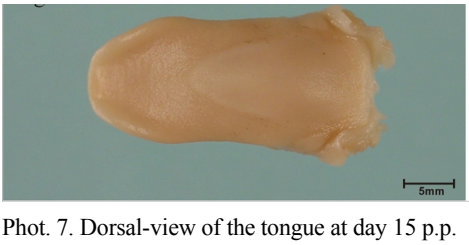
At day 1 p.p. a further increase in the length and width of the tongue could be observed on the apex, the body and the root of the tongue. The mean total length of the tongue was 20 mm. Thus the tongue was by 6 mm longer in comparison to the previous period in the life of the rabbit (Tab. 1). The weight of the tongue increased by 0.30 g (Tab. 2). The forming torus of the tongue was more visible than in the previous period in the life of the rabbit (Phot. 5 and 6).



Phot. 5. Dorsa-view of the tongue at day 1 p.p.
Fot. 5. Widok grzbietowy języka w 1. dniu p.p.

Phot. 6. Side-view of the tongue at day 1 p.p.
Fot. 6. Widok boczny języka w 1. dniu p.p.

At day 15 p.p. the width of the apex and the root was still bigger than the length of these parts of the tongue. The mean length of the tongue as a whole increased by 9 mm. The biggest increase was found for the length of the body of the tongue, while it was smallest for the root of the tongue (Tab. 1). The weight of the tongue increased by 1.11 g (Tab. 2). The torus of the tongue in the anterior part narrowed. A shallow median sulcus of the tongue was visible (Phot. 7 and 8).

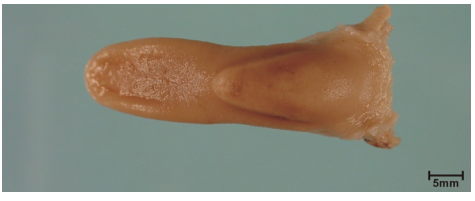


Phot. 7. Dorsal-view of the tongue at day 15 p.p.
Fot. 7. Widok grzbietowy języka w 15. dniu p.p.

Phot. 8. Side-view of the tongue at day 15 p.p.
Fot. 8. Widok boczny języka w 15. dniu p.p.

In the successive day 30 of postnatal life it was found that the width of the apex of the tongue was bigger than its length. The longest and at the same time widest part of the tongue was the body of the tongue, while the shortest and the narrowest part was its root (Tab. 1). The weight of the tongue in comparison with day 15 p.p. increased by 2.34 g (Tab. 2).

At day 30 p.p. the tongue in the rabbit resembled in its shape the tongue of an adult animal. The torus of the tongue was well-visible, similarly as the median sulcus (Phot. 9 and 10).

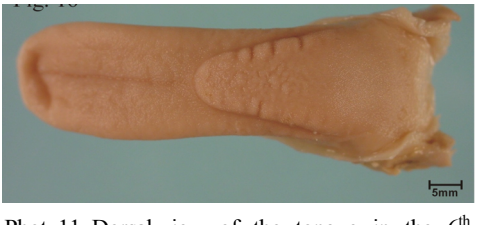


Phot. 9. Dorsal-view of the tongue at day 30 p.p.
Fot. 9. Widok grzbietowy języka w 30. dniu p.p.

Phot. 10. Side-view of the tongue at day 30 p.p.
Fot 10. Widok boczny języka w 30. dniu p.p.

In the 6th month of postnatal life the tongue of rabbits was 65 mm in length. The tongue was over 7 times longer than at day 22 of prenatal development. The torus of the tongue and the median sulcus were completely formed. The biggest increment in length was recorded for the body of the tongue, while the biggest increment in width was found for its root (Tab. 1). The weight of the tongue increased by as much as 5.62 g (Tab. 2).

The tongue observed in the 6th month of life was completely formed. The torus of the tongue was well-visible, which widened caudally. The median sulcus was marked and extended at the apex and the body of the tongue, ending in front of the torus of the tongue (Phot. 11 and 12).



Phot. 11. Dorsal-view of the tongue in the 6th month p.p.
Fot. 11. Widok grzbietowy języka w 6. miesiącu p.p.

Phot. 12. Side-view of the tongue in the 6th month p.p.
Fot. 12. Widok boczny języka w 6. miesiącu p.p.

DISCUSSION

Based on the conducted analyses it may be stated that the formation of the tongue in the rabbit is a long-term and complex process. In the period from day 15 p.c. to the 6th month p.p. numerous structural and metric changes were found in the examined organ.

Primordia of the tongue, i.e. two lateral lingual swelling, tuberculum impar and copula (hypobranchial eminence) contribute to the formation of the tongue in animals and

humans [Bartel 2002, Rüsse and Sinowatz 1991, Sadler 1993, Zietzschmann and Krölling 1955]. The tongue of the rabbit was formed on the basis of its primordia starting as early as day 15 p.c.

A significant finding was the determination of the prenatal age in the rabbit, at which primordia of individual lingual papillae were identified. It results from analyses that the earliest, i.e. already at day 15 p.c. circumvallate papillae start to develop, which belong to the category of gustatory papillae. At day 18 p.c. the presence of primordia of successive gustatory papillae was observed, i.e. fungiform papillae. At day 22 of prenatal development primordia of the third type of gustatory papillae appeared, i.e. those of foliate papillae as well as primordia of mechanical papillae, such as filiform papillae. The order of the formation of lingual papillae in the rabbit is similar to that in the other analyzed animal species, e.g. in the mouse [Iwasaki et al. 1996], rat [Iwasaki et al. 1997] and human [Witt and Reutter 1997]. It is generally known that the development of gustatory papillae is affected by sensory innervations [Whitehead and Kachele 1994]. Sensory innervation also has an effect on the development of taste buds. Evidence for this fact was supplied by research results reported by other authors [Ganchrow and Ganchrow 1989, Nakashima et al. 1990].

The shape and structure of the first forms of primordia of lingual papillae in the rabbit differ from those observed in adult animals [Kulawik and Godynicki 2006, 2007a, 2007b]. Primordia of lingual papillae shown in the rabbit at initial stages of development resemble primordia of papillae observed by other authors [Ahpin et al. 1989, Fujimoto et al. 1993, Jitpukdeebodinntra et al. 2002].

Statistical analysis of measurable traits of the tongue in the period from day 22 p.c. to the 6th month p.p. showed that in all examined periods the longest and widest parts of the tongue was the body of the tongue. Until day 1 p.p. the length of the apex and the root was identical, while after that period the apex was longer than the root of the tongue. The width of the apex exceeded the width of the root until the 6th month p.p., when equal numerical values describing that trait were recorded. Probably such results of analyses are connected with the development of the viscerocranium, the oral cavity and teeth and indirectly with the type of food consumed by rabbits – first liquid and then solid.

An increase in the weight of the tongue during prenatal development was slight. Only after birth its bigger increase was found. The biggest increment in weight was recorded between day 30 p.p. and the 6th month p.p.

Due to a lack of information on changes in metric characters of the tongue in the pre- and postnatal period in other animal species it is not possible to confront results obtained in this study.

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ROZWÓJ JĘZYKA U KRÓLIKA (*ORYCTOLAGUS CUNICULUS F. DOMESTICA*) ORAZ KOLEJNOŚĆ FORMOWANIA SIĘ BRODAWEK JĘZYKOWYCH W ŻYCIU PRE- I POSTNATALNYM

Streszczenie. Badanych było 129 języków, które pobrano poselekcyjnie od królików (*Oryctolagus f. cuniculus f. domestica*), będących w wieku od 15. dnia rozwoju prenatalnego p.c.) do 6. miesiąca życia postnatalnego (p.p.).
Język uformowany był na bazie swoich zawiązków już w 15. dniu p.c. Wtedy też stwierdzono pierwsze zawiązki brodawek językowych, jakimi były zawiązki brodawek okolonych. W 18. dniu p.c. odnotowano obecność zawiązków brodawek grzybowatych, a w 22. dniu p.c. zawiązki brodawek liściastych i nitkowatych. Na powierzchni grzbietowej języka w czasie rozwoju uformował się wał i bruzda pośrodkowa, która kończyła się przed wałem języka. Język królika zwiększył swoją długość od 22. dnia p.c. do 6. miesiąca p.p. z 9 do 65 mm. W tym czasie masa języka wzrosła z 0,05 do 9,59 g.
Kształtowanie się ostatecznej formy języka jest procesem długotrwałym i zachodzi zarówno w okresie życia prenatalnego, jak i postnatalnego. Najpierw pojawiają się zawiązki brodawek smakowych, a dopiero później zawiązki brodawek mechanicznych.

Słowa kluczowe: język, rozwój, brodawki

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ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Medicina Veterinaria 8(4) 2009, 27-32*

INVASION OF *LERNAEA CYPRINACEA* IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Małgorzata Bednarska, Michał Bednarski, Zenon Sołtysiak, Ryszard Polechoński

Wrocław University of Environmental and Life Sciences

Abstract. Our paper describes case of lerneosis in rainbow trout (*Oncorhynchus mykiss*). Lerneosis is disease of fish caused by *Lernaea cyprinacea.* This species is a cosmopolitan ectoparasite, worldwide distributed. Invasion by *L. cyprinacea* were rare described in Poland. This parasite is highly adapted to a parasitic life. The life cycle of *L. cyprinacea* is composed of nine stages. After mating males (mature stage) subsequently die and mated females attach to the body surfaces of host fish and then passing trough the skin to the muscles. Infections of this parasite can cause localized inflammation and ulceration at the area of attachment and are entrance of secondary bacterial and fungal infections. Massive invasion in fish can cause serious economic problems.

Key words: fish parasites, *Lernaea cyprinacea,* rainbow trout

INTRODUCTION

Lerneosis is disease of fish caused by anchorworm, an parasitic species of *Copepoda* belong to family *Lernaeidae*. The most common species infecting freshwater fish in Europe among the lerneaeid copepod is *Lernaea cyprinacea*. Invasion by *L. cyprinacea* were described in more then 100 fish species, belong to 25 different families [Kabata 1979]. However, this species is the most commonly observed in crucian carp (*Carrassius carassius*), gold fish (*Carassius auratus*) and common carp (*Cyprinus carpio*) [Antychowicz 2003, Jara and Chodyniecki 1999, Prost 1994].

L. cyprinacea is a cosmopolitan species, and occur in Europe, Asia, Africa, North America, Japan and Israel [Jara and Chodyniecki 1999, Kabata 1979]. This parasite has been introduced into South America and Australia with common carps [Piasecki et al. 2004]. The infestation of fish by this lerneaeid copepod in Poland is generally rare and usually described in Pomeranian, Masovian and Masurian region [Grabda 1955].

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Life cycle of *L. cyprinacea* is composed of nine stages. Following three naupliar stages are five copepoid stages and one adult stage – cyclopoid. Naupliar are free living forms, and copepodid and cyclopoid stage require fish host. After mating males (cyclopoid stage) subsequently die and mated females attach to the body surfaces of host fishes and then passing trough the skin to the muscles. Adult females of *L. cyprinacea* are highly adapted to a parasitic life. Anatomical modification to a parasitic model of life exhibit changes of thorax and cephalothorax. Thorax lost external evidence of segmentation, characteristic for subclass *Copepoda*. The anchors, by which the parasites attach itself to its host, are series of outgrowths on the cephalothorax. The anchors are cylindrical with a pair of bigger, ramified, Y-shaped or T-shaped dorsal processes and second smaller, unramified ventral pair. Adult females are 12–16 mm long and develop two egg sacs with 300–700 eggs [Grabda 1955, 1963].

The temperature of water plays an important role in transmission of the *L. cyprinacea.* The optimum temperature for reproduction and life cycle appears to be 23–30ºC and the development takes 16,5–20 days. *L. cyprinacea* stop its life cycle when temperature of water drops below 14ºC. Mated females survive winter attached to the body of fish. Two generations during one year are observed in Poland [Grabda 1955, 1963]. However, even ten generations during one year can be observed in warm climate [Piasecki et al. 2004].

L. cyprinacea is feed on fish blood and tissue debris. Invasion destroys scales, skin, muscles, and penetration of the fish body formats deep ulcers, abscesses or fistulas. Anchorworms can penetrate body cavity and abdominal organs in small fish. Invasions in fish can cause serious economic problems. Heavy parasitosis can be the cause of mass death of fish and secondary bacterial or fungal infections [Grabda 1955, 1963].

MATERIAL AND METHODS

Mass die of rainbow trout (*Oncorhynchus mykiss*) was observed in August 2007 in a recreation farm in Lower Silesia. Fish were husbandry in two ponds of 45 ar, characterized by slow water rate flow, and water temperature was 19,5ºC. Depth of ponds was approximately 3 m. Fish were stocked during the period 2006–2007. There was mixed culture of rainbow trout (*Oncorhynchus mykiss*) with different species: carp (*Cyprinus carpio*), tench (*Tinca tinca*), grass carp (*Ctenopharyngodon idella*), roach (*Rutilus rutilus*), carp bream (*Abramis brama*), northern pike (*Esox lucius*) and european perch (*Perca fluviatilis*). Fish were bought from different fish farms.

Ten rainbow trouts caught by fishing line from described ponds were examined. The average size fish caught range from 22 to 26 cm and weigh were ranging from 145 to 172 g. Parasites were collected using dissecting pens and preserved in 70% ethanol. Samples of the skin and muscle tissue surrounding the parasites were taken to the histopathological examination. The samples were fixed in formaldehyde solution, embedded in paraffin, cut into thin shavings and then H-E (hematoxillin and eosin) staining were undertaken.

RESULTS

In the macroscopic examinations large numbers (32–56) of parasites belong to family *Lernaeidae* were found (Phot. 1). All the parasites found during this survey were identified as females of *L. cyprinacea*, showing a length range of 10–14 mm and usually develop two egg sacs. In the examinations of the rainbow trout, edema of skin, hyperemia, lack or destroys scales were found in places where the parasites attach to its host. Cutaneous lesions were about 4–6 mm in diameter. Large, fuse skin lesions were formed in the places, where severe anchorworms attach closely to each other. In many sections healing ulcers were observed, heavily colonized bay a mixed bacterial flora.

Phot. 1. Invasion of *Lernaea cyprinacea* in rainbow trout (*Oncorhynchus mykiss*)
Fot. 1. Inwazja *Lernaea cyprinacea* u pstrąga tęczowego (*Oncorhynchus mykiss*)

The histological examination of samples taken from surrounding *L. cyprinacea* showed infiltration of inflammatory mononuclear cells (lympho-histiocytes) (Fig. 1). The attachment areas were surrounded by small areas of hemorrhage with hemolyzed erythrocytes and hemosyderin loaded macrophages. Lesions of epidermis and dermis exhibited focuses of moderate to severe granulomatous inflammation (Fig. 2). In most sections the inflammation involved only epidermis and dermis, but few transmular inflammation expended into the focal muscular layer with inflammatory cells found within endomysial connective tissue. Inflammatory infiltration in muscle tissue caused muscle parenchymatous and hyaline degeneration and muscle fiber atrophy (Fig. 3). The inflammatory infiltrate was composed predominantly of lymphocytes and histiocytes, but focal accumulation of small numbers of eosynophilic granulocytes and fibroblasts was observed.

There is no successful method to eradicated *L. cyprinacea* in natural ponds and to get rid of adult stage. There is no medicament for treatment of this parasites that is allowed in Poland as well. There was no decision about treatment because of this reason. Mass die of rainbow trout was observed until the end of summer, caused die off of this species in both ponds.

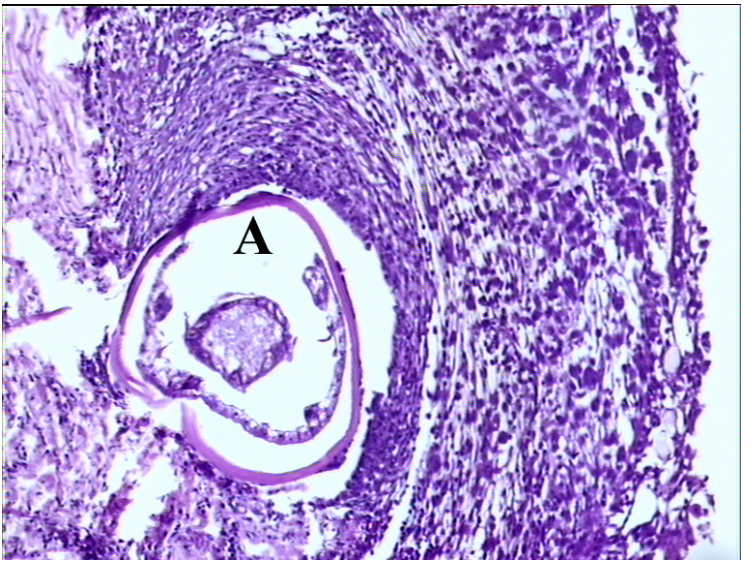

Fig. 1. The histopathological section of cutaneous lesions with *L. cyprinacea* (A). The attachment area is surrounded by inflammatory mononuclear and polynuclear cells (H-E, magnification 100x)

Ryc. 1. Obraz histopatologiczny zmian skórnych wywołanych przez *L. cyprinacea (A).* Miejsce zakotwiczenia pasożyta jest otoczone przez naciek zapalny komórek jedno- i wielojądrzastych (barwienie H-E, powiększenie 100x)

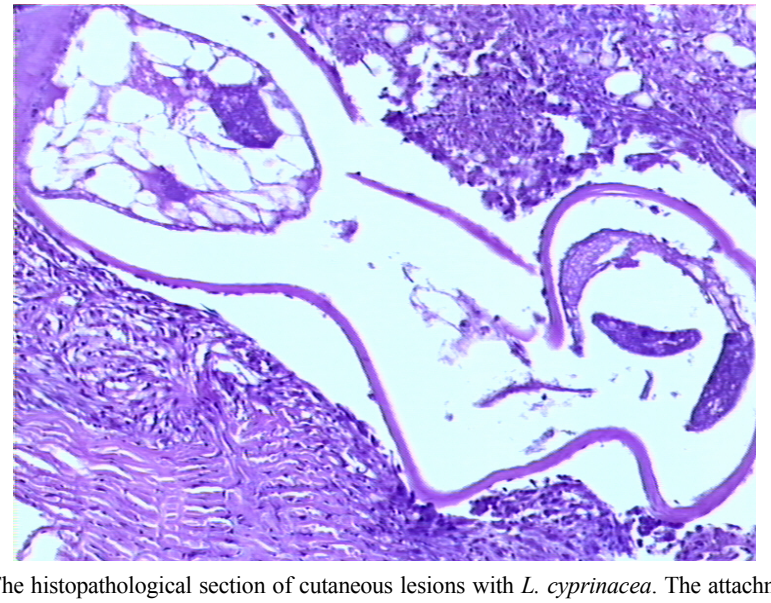

Fig. 2. The histopathological section of cutaneous lesions with *L. cyprinacea*. The attachment area is surrounded by moderate to severe granulomatous inflammation (H-E, magnification 100x)

Ryc. 2. Obraz histopatologiczny zmian skórnych wywołanych przez *L. cyprinacea* (A). Miejsce zakotwiczenia pasożyta jest otoczone przez silny naciek granulocytarny (H-E, powiększenie 100x)

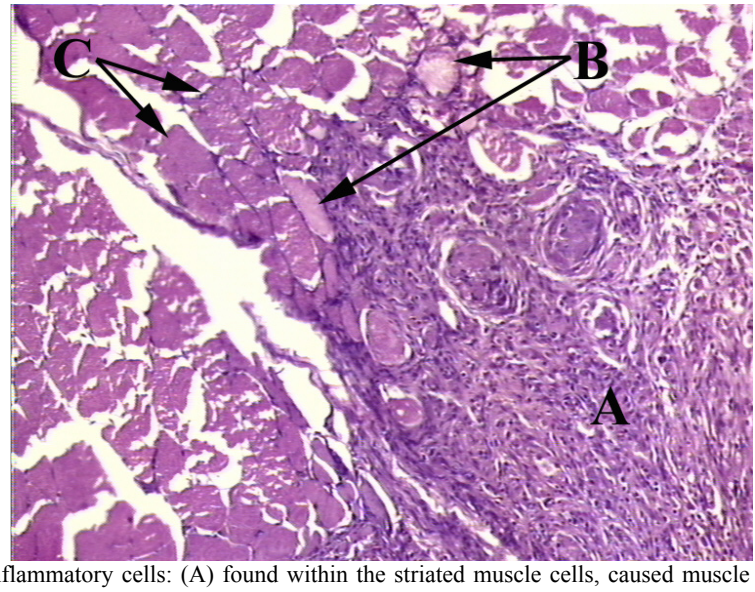

Fig. 3. Inflammatory cells: (A) found within the striated muscle cells, caused muscle parenchymatous and hyaline degeneration (B) and muscle fiber atrophy (C). (H-E, magnification 100x)

Ryc. 3. Nacieki komórek zapalnych: obejmujący tkankę mięśniową (A) i powodujący zmiany zwyrodnieniowe miąższowe i szkliste włókien mięśniowych (B) oraz ich zanik (C)

DISCUSSION

Invasion of *Lernaea* sp. in rainbow trout were described very rare in literature. However, Grabda [1955a, 1956] indicates that rainbow trout is very sensitive for invasion of this parasite. Flow rate is critical for life cycle of *L. cyprinacea.* Heavy parasitosis is usually observed in ponds, characterized by slow water flow and elevated water temperature [Berry et al. 1991, Grabda 1955a, 1956].

In our case report, type of fish husbandry: mixed fish species, under crowded culture conditions, slow water flow and relatively high water temperature – made good environment for *L. cyprinacea*. Moreover, described conditions were not proper for rainbow trout aquaculture. Exposure to these factors together with heavy parasitosis caused mass die of fish.

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INWAZJA LERNAEA CYPRINACEA U PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS*)

Streszczenie. W niniejszym artykule opisano przypadek inwazji *Lernaea cyprinacea* u pstrąga tęczowego (*Oncorhynchus mykiss*). Pasożyt ten jest kosmopolitycznym gatunkiem, występującym również w Polsce. Jest przystosowany do pasożytniczego trybu życia. Cykl rozwojowy składa się z dziewięciu stadiów. Samce po osiągnięciu dojrzałości płciowej kopulują i giną, natomiast samice wczepiają się w skórę ryby, tracąc zdolność poruszania się. Inwazja *Lernaea cyprinacea* prowadzi do uszkodzenia skóry i mięśni oraz powstania stanów zapalanych i owrzodzeń w miejscu przyczepu, a także wtórnych zakażeń bakteryjnych i grzybiczych. W przypadku występowania silnych inwazji *L. cyprinacea* może prowadzić do istotnych strat ekonomicznych.

Słowa kluczowe: pasożyty ryb, *Lernaea cyprinacea,* pstrąg tęczowy

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ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Medicina Veterinaria 8(4) 2009, 33-48*

THE INFLUENCE OF THE SELECTED CALCIUM CHANNEL BLOCKERS ON THE UTERINE CONTRACTION ACTIVITY IN RATS IN THE *IN VITRO* CONDITIONS

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Abstract. The study has been conducted on 20 Bufflo female rats aged 4–6 months, from which the uterus strips have been taken directly after slaughter. Isolated uterus strips were placed in 4 automatic water bath chambers. After recording of spontaneous contraction activity of the isolated strip, calcium channel blockers i.e. diltiazem, nifedypine, verapamil were added into the bath organ. Doses of the preparations were determined experimentally. The experiments proved that an isolated uterus strip showed spontaneous periodical contraction activity for about 8-12 hours. An average contraction frequency amounted to 1.36 ± 0.33 contractions per minute, and strength of a single contraction was on average 2.7 ± 0.7 grams. Administration of oxytocin into the incubation bath organ increases the frequency (by 44.85 ± 13.42%) and strength (by 39.5 ± 18.33%) of the spontaneous uterine contraction activity. Administration of the calcium channel blockers: diltiazem, nifedypine, verapamil into the incubation bath organ causes a decrease or atony of the frequency and strength of the uterus muscular coat contractions depending on concentration of an active substance of the preparation. Nifedypine is the strongest calcium channel blocker. Blocking of the calcium channels with a 2.8×10^{-8} mol/l dose of nifedypine resulted in a decrease in frequency by 40 ± 9.86%, whereas a dose ten times higher (2.8×10^{-7}) caused total atony of the spontaneous uterine contraction activity. Administration of oxytocin into the incubation bath organ after the prior total blocking of the Ca^{2+} channels does not result in an increase in the isolated strip contraction activity.

Key words: calcium channels, nifedypine, diltiazem, verapamil, uterus contractility, rat

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INTRODUCTION

Calcium plays a key role in the living organisms. It is the main constituent of bones, teeth, and nails. This element takes part in many processes which are necessary for proper organism functioning. It participates in many processes including muscular contraction and decontraction mechanics, and the blood clotting process. It is capable of enzyme activation and inhibition and it takes part in cell membrane permeability. Calcium penetrates through inside the cells by means of calcium channels. This transport can be inhibited by drugs called calcium channel blockers. The blockers comprise nifedypine, diltiazem and verapamil but are not limited to them. They remove a flux of calcium ions in heart tissues, smooth muscles of blood vessels, and somatic muscles [Opie 1984, Allert and Adams 1987]. The blockers have applications mainly in supraventricular tachyarrhythmia treatment, hypertension therapy, and angina pectoris therapy [Rang et al. 2001]. The work presented makes an attempt to determine an influence of the selected calcium channel blockers and oxytocin (diltiazem, nifedypine, verapamil) on the spontaneous contraction activity of the rat uterus muscular coat in the *in vitro* conditions and to compare strength of the above-mentioned blockers on inhibiting the spontaneous uterus activity based on differences in concentration of the preparations in the incubation bath.

MATERIALS AND METHODS

The study has been conducted on 20 Bufflo female rats aged 4–6 months. The animals were kept in metal cages with 3 rats per cage and fed LMS, whole portion mixture for rodents, *ad libitum*. The experiments were carried out with the Local Ethical Board's consent (application No. 317/04. opinion No. 68/04). The animals were put to halothane narcosis and after death (after their decapitation) their uterus strips 1.5–2 cm long were taken for examination. The strips were placed in 4 automatic bath chambers of 20 cm^3 capacity. The Krebs-Henseleit buffer with the composition of: NaCl – 118 mM; KCl – 4.7 mM; $CaCl_2$ – 2.5 mM; $MgSO_4$ – l.6 mM; $NaHCO_3$ – 24.3 mM; KH_2PO_4 – l.18 mM; glucose 5.6 mM was used as incubation environment.

The strips incubation was conducted at the temperature of 37°C, while the oxygen and carbon dioxide gas mixture: 95% O_2 and 5% CO_2, was added so that its pH remained within 7.3–7.5. The uterine contractions were recorded by isotonic transducers (Letica Scientific Instruments) connected to bridge amplifiers (BridgeAmp, ADInstruments, Australia) with a four-channel data acquisition system (PowerLab/400, ADInstruments) connected to a Macintosh computer. After the 30 minute recording of spontaneous myometrial contraction activity, the following calcium channel blockers were added into the bath chamber:

1. Diltiazem hydrochloride (SIGMA) – calcium channel blocker, benzodiazepine derivative, water-soluble;
2. Nifedypine (SIGMA) – calcium channel blocker, dihydropiridine derivative, DMSO-and-ethanol-soluble;
3. Verapamil hydrochloride (SIGMA) – calcium channel blocker, phenylalkyloamine derivative, water-soluble.

Before and after the calcium channel blockers were administered, a dose of 0.01 mass units (determined experimentally) of oxytocin was added to the incubation bath in order to check an answer of the uterus muscular coat to the hormone.

Doses of the preparations were determined experimentally through preparation of tenfold dilution of the preparations: 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml, 0.001 mg/ml, and 0.0001 mg/ml. On measuring the dose, the preparation was added to the incubation bath ranging from the lowest dilution in the dose concentration system (that is without rinsing of the bath between the subsequent applications of the doses to the bath) to the evident effect on the recording. The lowest preparation concentration resulting in the desired effect was accepted as the experimental dose and was confirmed in a number of the next experiments.

The number of contractions per min (contractility frequency) and the contractions strength given in grams were taken into consideration while compiling the obtained results [Czerski et al. 2004]. The study results were compiled in the Microsoft Excel 2000 and put to statistical analysis by using Student's t-test and the single agent analysis of variance (ANOWA) for separate variables.

RESULTS

SPONTANEOUS RAT UTERUS MUSCULAR COAT CONTRACTION ACTIVITY IN *IN VITRO* CONDITIONS

An isolated strip of rat uterus hung in the automatic water bath showed spontaneous contractility for about 8 to 12 hours. The average contractility frequency amounted to 1.36 ± 0.33 contractions per minute and the average contraction strength was at a level of 2.7 ± 0.7 grams (Fig. l). The application of oxytocin into the incubation bath organ at the same time in the experimentally stated dose (0.01 i.u.), caused an increase in the contractility frequency by 44.85 ± 13.42% ($p \leq 0.001$, $n = 20$). The contractility frequency amounted to 1.97 ± 0.47 contraction per min. after oxytocin was added. A significant increase in the average strength of single contraction by 39.5 ± 18.33% ($p \leq 0.001$, $n = 20$) was also noted, and the strength achieved a value of 3.76 ± 0.97 grams (Fig. 2 and Graph l and 2). After the application of oxytocin into the incubation bath smooth muscle tonus of the uterus increased by 0.88 ± 0.15 grams (Fig. 2). Phases of the sexual cycle did not influence the study results obtained as no statistically significant changes were noticed in recording of spontaneous contractility of the uterus muscular coat strips coming from the animals in different cycle phases.

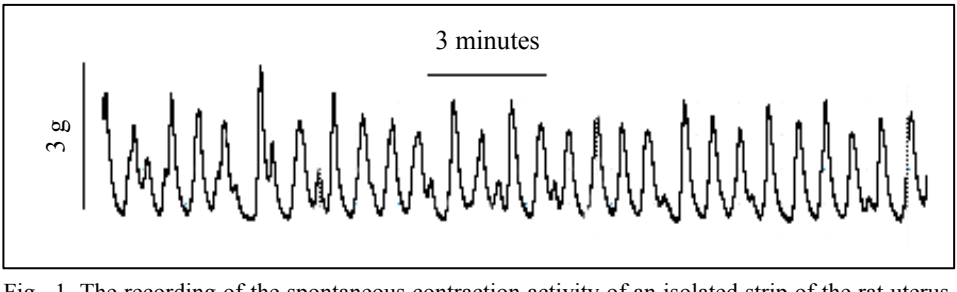


Fig. 1. The recording of the spontaneous contraction activity of an isolated strip of the rat uterus in the *in vitro* conditions

Ryc. 1. Zapis spontanicznej aktywności skurczowej izolowanego skrawka macicy szczurów w warunkach *in vitro*

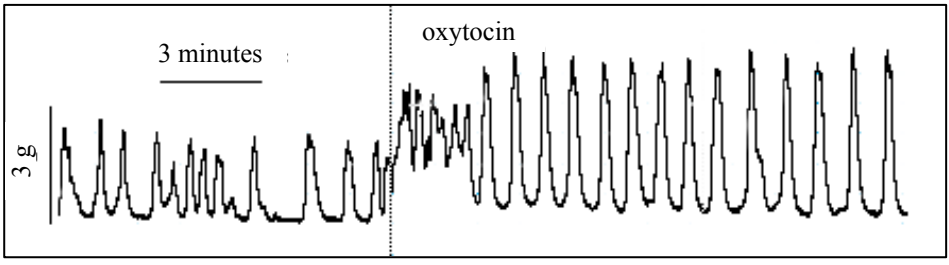
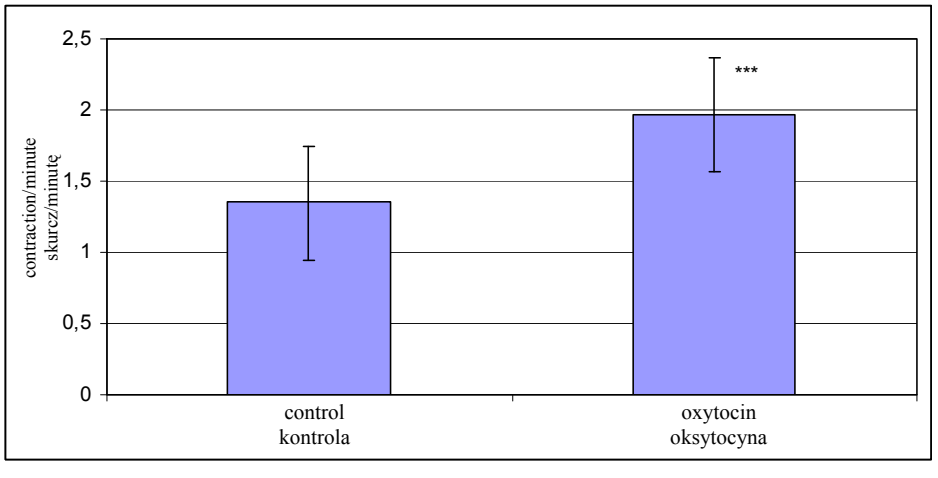


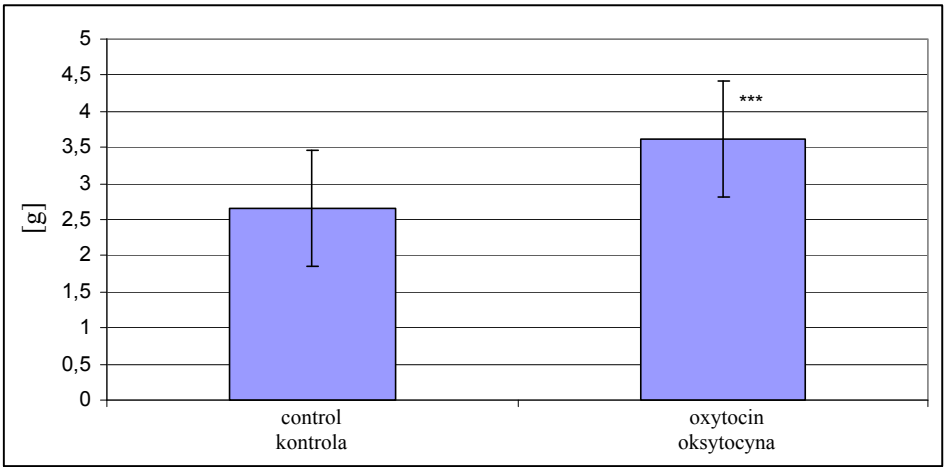
Fig. 2. The recording of the spontaneous uterus contractility after administration of oxytocin. Administration time is marked with a dotted line. A noticeable increase in contraction frequency (by 44.85%) and strength (by 39.5%) compared to the initial recording

Ryc. 2. Zapis kurczliwości mięśniówki macicy po podaniu oksytocyny. Czas podania zaznaczono przerywaną linią. Widoczny wzrost częstości (o 44,85%) i siły skurczów (o 39,5%) w porównaniu do zapisu wyjściowego



Graph l. Frequency of the spontaneous contraction activity before and after administration of oxytocin in a dose of 0.01 i.u. into the incubation bath organ. A noticeable increase in a contraction frequency amplitude after administration of oxytocin by 44.85 ± 13.42% compared to the control. x ± SD (n=20) has also been indicated on the Graph. *** $p < 0.001$

Wykres l. Częstotliwość spontaniczna aktywności skurczowej przed i po podaniu oksytocyny w dawce 0,01 j.m. do komory inkubacyjnej. Widoczny wzrost amplitudy częstości skurczów po podaniu oksytocyny o 44,85 ± 13,42% w porównaniu z kontrolą. Na wykresie zaznaczono również x ± SD (n = 20). *** $p < 0,001$



Graph 2. Strength of the spontaneous contraction activity before and after administration of oxytocin in a dose of 0.01 i.u. into the incubation bath organ A noticeable increase in a single contraction strength after administration of oxytocin by 39.5 ± 18.33% compared to the control. X ± SD (n = 20) has also been indicated on the Graph. *** $p < 0.001$

Wykres 2. Siła spontanicznej aktywności skurczowej przed i po podaniu oksytocyny w dawce 0,01 j.m. do komory inkubacyjnej. Widoczny wzrost siły pojedynczego skurczu po podaniu oksytocyny o 39,5 ± 18,33% w porównaniu z kontrolą. Na wykresie zaznaczono również X ± SD (n = 20). *** $p < 0,001$

IMPACT OF DIFFERENT NIFEDYPINE CONCENTRATIONS (CALCIUM CHANNEL BLOCKER) ON THE SPONTANEOUS RAT UTERUS MUSCULAR COAT CONTRACTION ACTIVITY

Blocking of the calcium channels with nifedypine (calcium channel antagonist) in a dose of 2.8×10^{-8} mol/l caused a decrease in contraction frequency by 40±9.86% up to a value of 0.81 ± 0.19 contraction per min ($p \leq 0.001$, n = 10) and contraction strength by 11.15 ± 2.82% up to a value of 2.39±0.62 grams ($p \leq 0.05$, n = 10), whereas a ten-times higher dose ($2,8 \times 10^{-7}$ mol/l) resulted in a total lack of uterus muscular coat spontaneous contractility (atony) (Fig. 3). The decrease in contraction observed after the application of nifedypine into the incubation bath organ is proportional to the preparation concentration in the incubation bath organ, which is illustrated by Graphs 3 and 4. The application of oxytocin into the incubation bath organ in a dose of 0.01 i.u. at the same time did not cause an increase in the contractility activity of the isolated strip, which may have been observed in the control experiments (Fig. 4).

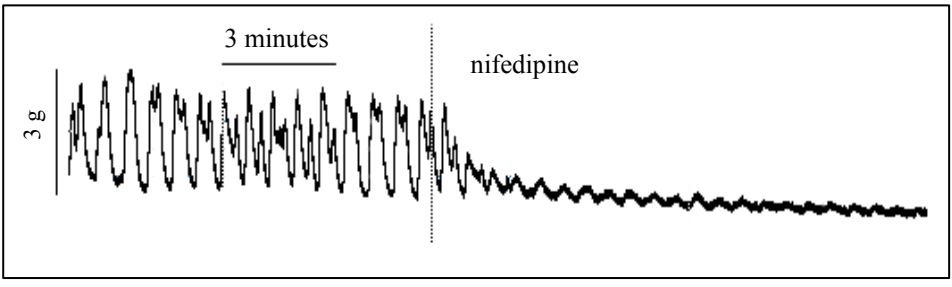


Fig. 3. The recording of the spontaneous uterus contractility after administration of nifedypine in a dose of $2.8x10^{-7}$mol/l. Administration time is marked with a dotted line. A noticeable drop in contractility of an isolated strip of the uterus muscular coat caused by the calcium channel blocking

Ryc. 3. Zapis aktywności skurczowej mięśniówki macicy po podaniu nifedypiny w dawce $2,8x 10^{-7}$mol/l. czas podania zaznaczono pionową przerywaną linią. Widoczny spadek kurczliwości wyizolowanego skrawka mięśniówki macicy spowodowany zablokowaniem kanałów wapniowych

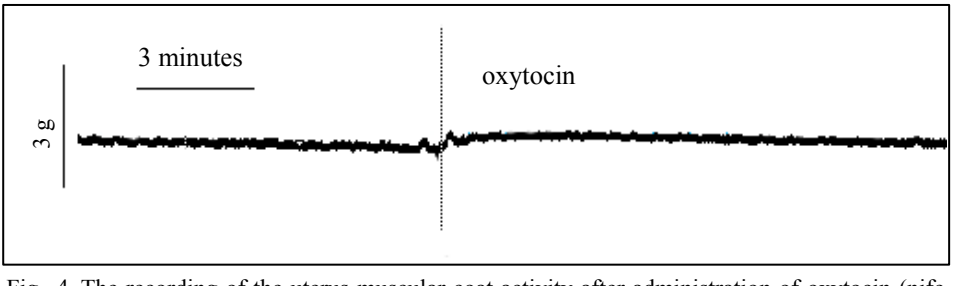
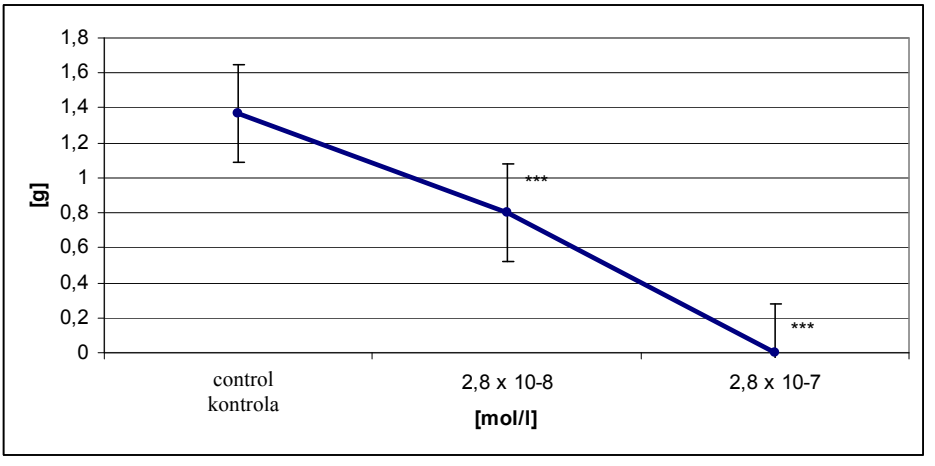


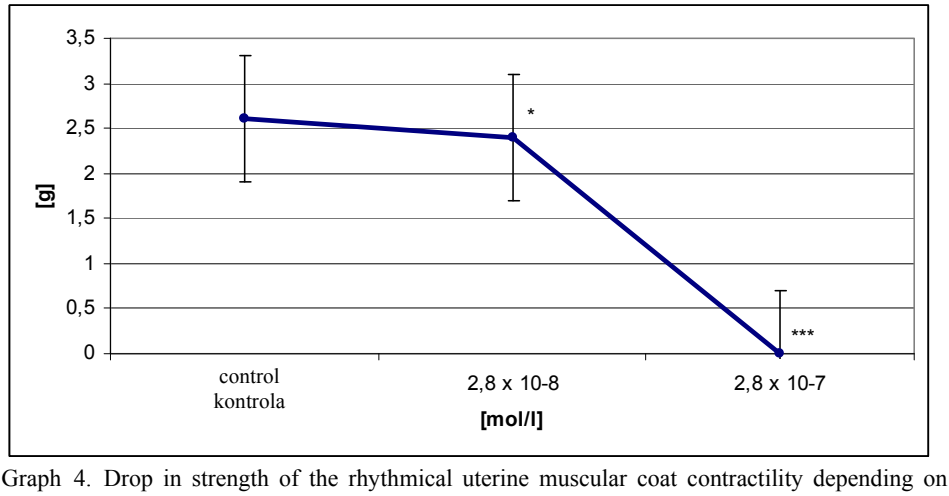
Fig. 4. The recording of the uterus muscular coat activity after administration of oxytocin (nifedypine in a dose of $2.8x10^{-7}$mol/l was administered into the bath organ before). Administration time is marked with a vertical dotted line. A noticeable lack of the stimulating effect of oxytocin caused by the total blocking of the calcium channels

Ryc. 4. Zapis aktywności mięśniówki macicy po podaniu oksytocyny (wcześniej do komory podano nifedypinę w dawce $2,8x10^{-7}$mol/l). Czas podania zaznaczono pionową przerywaną linią. Widoczny brak stymulującego wpływu oksytocyny spowodowany całkowitym zablokowaniem kanałów wapniowych



Graph 3. Drop in frequency of the rhythmical uterine muscular coat contractility depending on nifedypine concentration in the incubation bath organ. The noted drop proceeded as concentration of the active substance was growing. $x \pm SD$ (n = 10) has also been indicated on the Graph. *** $p \leq 0{,}001$

Wykres 3. Spadek częstotliwości rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji nifedypiny w komorze inkubacyjnej. Odnotowany spadek następował w miarę wzrostu stężenia substancji czynnej. Na wykresie zaznaczono również $x \pm SD$ (n = 10). *** $p \leq 0{,}001$



Graph 4. Drop in strength of the rhythmical uterine muscular coat contractility depending on nifedypine concentration in the incubation bath organ. The noted drop in strength of a single contraction proceeded as concentration of the active substance was growing. $x \pm SD$ (n = 10) has also been indicated on the Graph. *** $p \leq 0.001$ * $p \leq 0.05$

Wykres 4. Spadek siły rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji nifedypiny w komorze inkubacyjnej. Odnotowany spadek siły pojedynczego skurczu następował w miarę wzrostu stężenia substancji czynnej. Na wykresie zaznaczono również $x \pm SD$ (n = 10). *** $p \leq 0{,}001$ * $p \leq 0{,}05$

IMPACT OF DIFFERENT DILTIAZEM CONCENTRATIONS (CALCIUM CHANNEL BLOCKER) ON THE SPONTANEOUS RAT UTERUS MUSCULAR COAT CONTRACTION ACTIVITY

Blocking of the calcium channels with diltiazem (calcium channel antagonist) in a dose of 0.66 x $l0^{-4}$ mol/l caused a decrease in contraction frequency by 27.27 ± 6.34% up to a value of 0.98 ± 0.24 ($p \leq 0.001$, n = 10) and contraction strength by 27.58 ± 6.41% up to a value of 1.95 ± 0.50 ($p \leq 0.001$, n = 10), whereas a ten-times higher dose (0.66 x 10^{-3}mol/1) resulted in a total lack of uterus muscular coat spontaneous contractility (atony) (Fig. 5). The decrease in contraction observed after the application of diltiazem into the incubation bath organ is proportional to the preparation concentration in the incubation bath organ, which is illustrated by Graphs 5 and 6. The application of oxytocin into the incubation bath organ in a dose of 0.01 i.u. at the same time did not cause an increase in the contractility activity of the isolated strip, which may have been observed in the control experiments (Fig. 6).

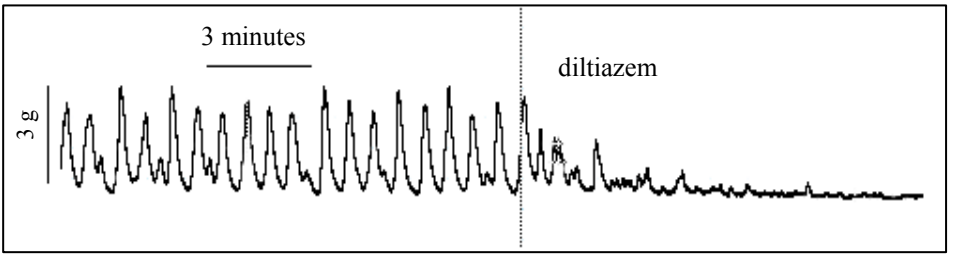


Fig. 5. The recording of the uterus muscular coat activity after administration of diltiazem in a dose of 0.66x10^{-3}mol/l. Administration time is marked with a vertical dotted line. A noticeable drop in contractility of an isolated strip of the uterus muscular coat caused by the calcium channel blocking

Ryc. 5. Zapis aktywności skurczowej mięśniówki macicy po podaniu diltiazemu w dawce 0,66x10^{-3}mol/l. Czas podania zaznaczono pionową przerywaną linią. Widoczny spadek kurczliwości wyizolowanego skrawka mięśniówki macicy spowodowany zablokowaniem kanałów wapniowych

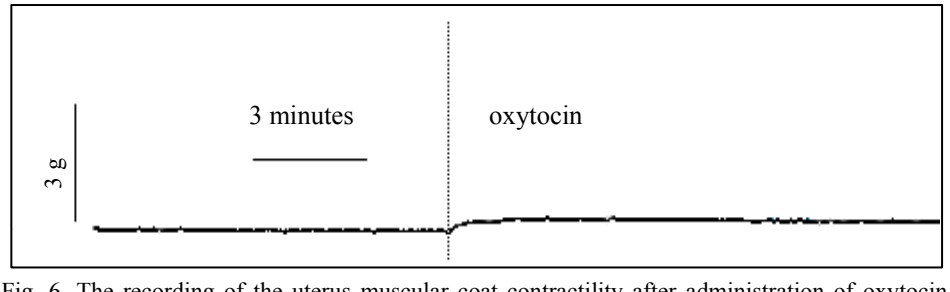
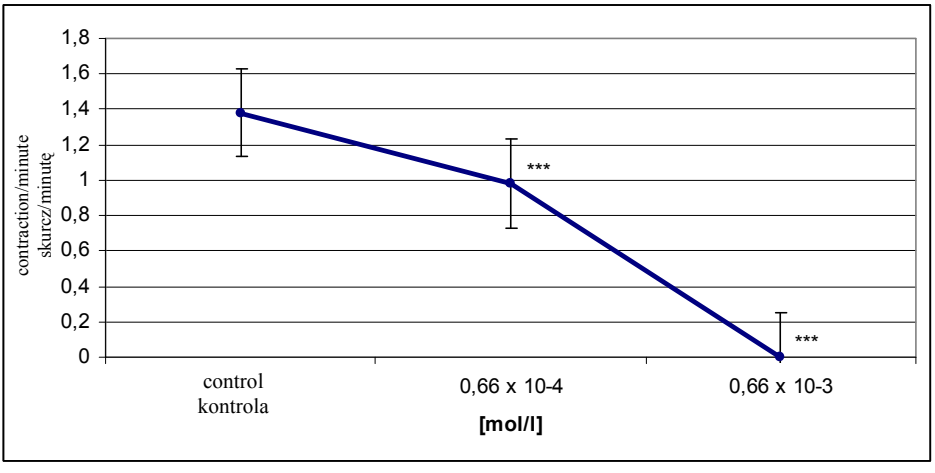


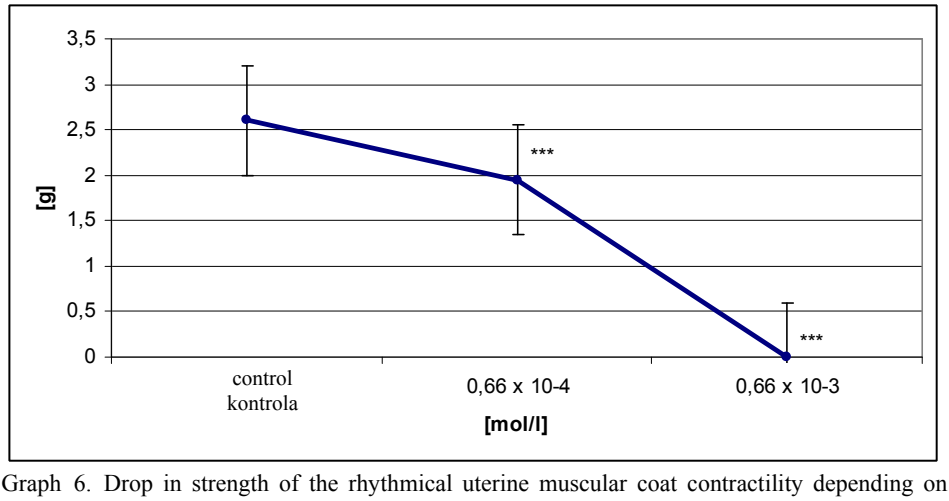
Fig. 6. The recording of the uterus muscular coat contractility after administration of oxytocin (diltiazem in a dose of 0.66x10^{-3}mol/l was administered into the bath organ before). Administration time is marked with a vertical dotted line. A noticeable lack of the stimulating effect of oxytocin caused by the total blocking of the calcium channels

Ryc. 6. Zapis kurczliwości mięśniówki macicy po podaniu oksytocyny (wcześniej do komory podano diltiazem w dawce 0,66x10^{-3}mol/l). Czas podania zaznaczono pionową przerywaną linią. Widoczny brak stymulującego wpływu oksytocyny spowodowany całkowitym zablokowaniem kanałów wapniowych



Graph 5. Drop in frequency of the rhythmical uterine muscular coat contractility depending on diltiazem concentration in the incubation bath organ. The noted drop proceeded as concentration of the active substance was growing. $x \pm SD$ (n = 10) has also been indicated on the Graph. *** $p \leq 0.001$

Wykres 5. Spadek częstotliwości rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji diltiazemu w komorze inkubacyjnej. Odnotowany spadek następował w miarę wzrostu stężenia substancji czynnej. Na wykresie zaznaczono również $x \pm SD$ (n = 10). *** $p \leq 0{,}001$



Graph 6. Drop in strength of the rhythmical uterine muscular coat contractility depending on diltiazem concentration in the incubation bath organ. The noted drop in strength of a single contraction proceeded as concentration of the active substance was growing. $x \pm SD$ (n = 10) has also been indicated on the Graph. ***$p \leq 0.001$

Wykres 6. Spadek siły rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji diltiazemu w komorze inkubacyjnej. Odnotowany spadek siły pojedynczego skurczu następował w miarę wzrostu stężenia substancji czynnej. Na wykresie zaznaczono również $x \pm SD$ (n = 10). ***$p \leq 0{,}001$

IMPACT OF DIFFERENT VERAPAMIL CONCENTRATIONS (CALCIUM CHANNEL BLOCKER) ON THE SPONTANEOUS RAT UTERUS MUSCULAR COAT CONTRACTION ACTIVITY

Blocking of the calcium channels with verapamil (calcium channel antagonist) in a dose of 0.5 x 10^{-4} mol/l caused a decrease in contraction frequency by 38.46 ± 7.54% up to a value of 0.83 ± 0.20 ($p \leq 0.001$, n = 10) and contraction strength by 51.85 ± 9.82% up to a value of 1.30 ± 0.33 ($p \leq 0.001$, n = 10), whereas a ten-times higher dose (0.5 x $l0^{-3}$ mol/l) resulted in a total lack of uterus muscular coat spontaneous contractility (atony) (Fig. 7). The decrease in contraction observed after the application of verapamil into the incubation bath organ is proportional to the preparation concentration in the incubation bath organ, which is illustrated by Graphs 7 and 8. The application of oxytocin into the incubation bath organ in a dose of 0.01 i.u. at the same time did not cause an increase in the contractility activity of the isolated strip, which may have been observed in the control experiments (Fig. 8).

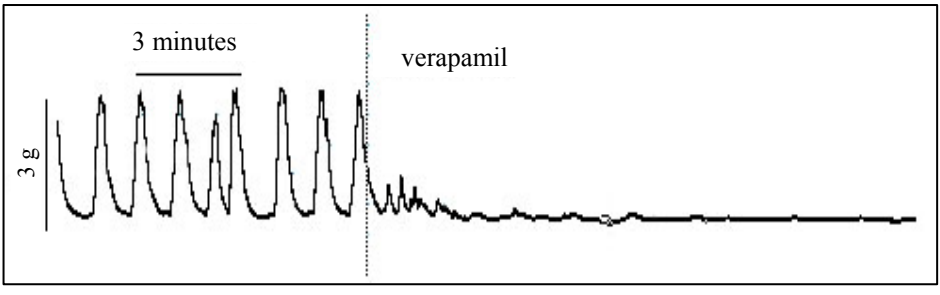


Fig. 7. The recording of the uterus muscular coat contraction activity after administration of verapamil in a dose of $0.5x10^{-3}$ mol/l. Administration time is marked with a vertical dotted line. A noticeable drop in contractility of an isolated strip of the uterus muscular coat caused by the calcium channel blocking

Ryc. 7. Zapis aktywności skurczowej mięśniówki macicy po podaniu werapamilu w dawce $0,5x10^{-3}$ mol/l. Czas podania zaznaczono pionową przerywaną linią. Widoczny spadek kurczliwości wyizolowanego skrawka mięśniówki macicy spowodowany zablokowaniem kanałów wapniowych

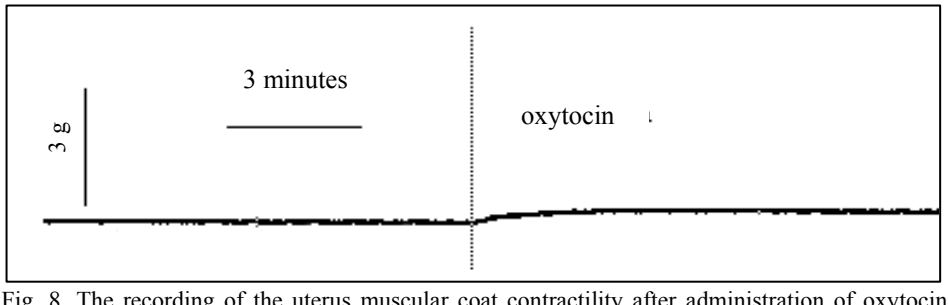
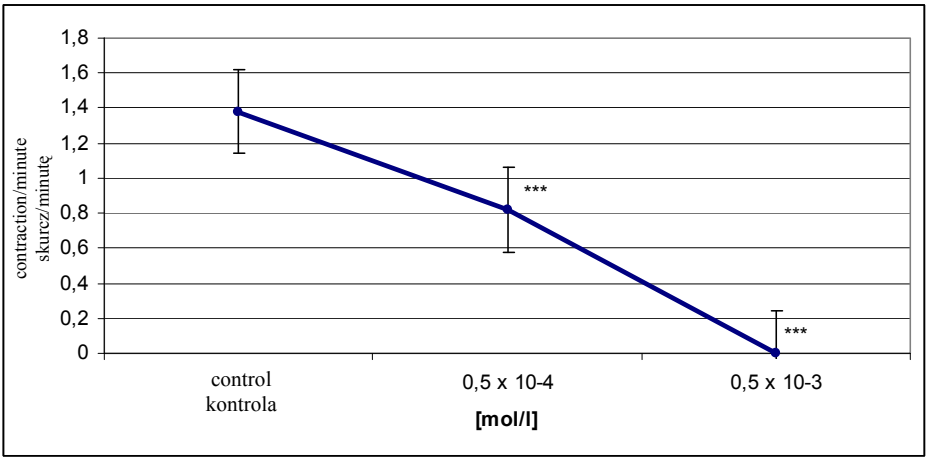


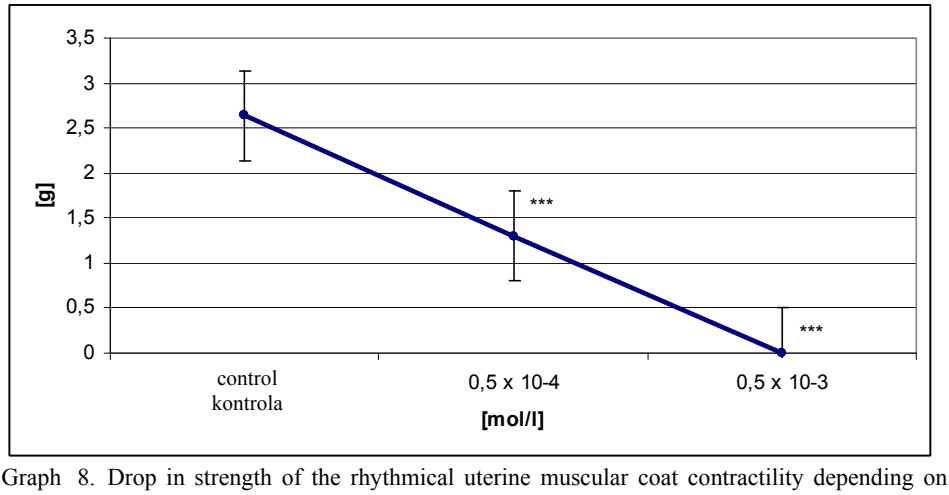
Fig. 8. The recording of the uterus muscular coat contractility after administration of oxytocin (verapamil in a dose of $0.5x10^{-3}$mol/l was administered into the bath organ before). Administration time is marked with a vertical dotted line. A noticeable lack of the stimulating effect of oxytocin caused by the total blocking of the calcium channels.

Ryc. 8. Zapis kurczliwości mięśniówki macicy po podaniu oksytocyny (wcześniej do komory podano werapamil w dawce $0,5x10^{-3}$mol/l). Czas podania zaznaczono pionową przerywaną linią. Widoczny brak stymulującego wpływu oksytocyny spowodowany całkowitym zablokowaniem kanałów wapniowych



Graph 7. Drop in frequency of the rhythmical uterine muscular coat contractility depending on verapamil concentration in the incubation bath organ The noted drop proceeded as concentration of the active substance was growing. $x \pm SD$ (n=10) has also been indicated on the Graph. *** $p \leq 0.001$

Wykres 7. Spadek częstotliwości rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji werapamilu w komorze inkubacyjnej. Odnotowany spadek następował w miarę wzrostu stężenia substancji czynnej. Na wykresie zaznaczono również $x \pm SD$ (n = 10). *** $p \leq 0{,}001$



Graph 8. Drop in strength of the rhythmical uterine muscular coat contractility depending on verapamil concentration in the incubation bath organ. The noted drop in strength of a single contraction proceeded as concentration of the active substance was growing. $x \pm SD$ (n = 10) has also been indicated on the Graph. *** $p \leq 0.001$

Wykres 8. Spadek siły rytmicznej kurczliwości mięśniówki macicy w zależności od koncentracji werapamilu w komorze inkubacyjnej. Odnotowany spadek siły pojedynczego skurczu następował w miarę wzrostu stężenia substancji czynnej. Na wykresie oznaczono również $x \pm SD$ (n = 10). *** $p \leq 0.001$

DISCUSSION

SPONTANEOUS CONTRACTION ACTIVITY

The isolated strips of rat uterus hung in the in the automatic water bath show spontaneous contraction activity for about 8 to 12 hours. During this time, contraction strength and frequency oscillated at a constant level: the average contractility frequency amounted to 1.36 ± 0.33 contractions per minute and the average single contraction strength amounted to 2.7 ± 0.7 grams. What can be concluded from the obtained study results is that the phases of sexual cycle did not have an influence on the contraction activity of the rat uterus muscular coat. No statistically significant changes were found in recording of spontaneous contractility of the uterus muscular coat strips taken from the animals in different cycle phases. It is consistent with the results obtained by Czerski et al. [2004a, b], which proved that periodical contraction activity of the isolated rat uterus strip lasted for about 8–12 hours. The average contractility frequency amounted to 1.15 ≤ 0.38 contractions per minute and the average single contraction strength amounted to 2.8 ± 0.45 grams and was directly proportional to a strip size [Czerski et al. 2004a, b]. Similarity of the results obtained may result from the fact that the incubation of isolated rat uterus strips was carried out in identical conditions.

IMPACT OF OXYTOCIN ON THE CONTRACTION ACTIVITY

The study has proved a stimulating effect of oxytocin on the spontaneous contraction activity of the rat uterus muscular coat. Administration of oxytocin into the incubation bath organ in a dose of 0.01 i.u. determined in the initial experiments caused both an increase in strength and frequency of contractions of the isolated uterus strips. The contractility frequency after the administration of oxytocin amounts to 1.97 ± 0.47 contractions per minute and the average strength of single contraction achieved a value of 3.76 ± 0.97 grams.

According to Zięba and Dejneka [1995] intravenous administration of oxytocin in a dose of 2 i.u. to sheep in an anestrus phase of the oestrus cycle without prior estrogen stimulation does not cause changes to the uterine contraction activity. Lack of response of the uterus muscular coat to the oxytocin stimulation may be caused by too low concentration of oxytocin receptors to evoke depolarization of the uterus muscle cells [Matthews et al. 1994, Zięba and Dejneka 1995]. However, an increase in the sheep uterine contraction activity after the administration of oxytocin can be observed after prior stimulation of the sheep with estrogens. It is very likely to be connected with sensitivity of the oxytocin receptors to oxytocin or with an increase in their concentration [Zięba and Dejneka 1995]. Administration of oxytocin results in an increase in frequency of spike potential discharges and a response time depends on a dose of oxytocin administered [Zięba and Dejneka 1995].

Oxytocin is considered as one of the strongest known agents which cause the uterus contractility [Szal et al. 1994]. Kawarabayashi et al. [1997] proved an influence of oxytocin on a change of length and width of the newly-isolated spindle cells of the rat uterus muscular coat. The observed uterus contractility occurs thanks to the oxytocin receptors situated on the surface of myocytes of smooth muscles. The study was carried out on day 15, 18 and 21 of pregnancy. The length of the isolated cells amounts, respectively, to 121 ± 8.1 μm on the 15th day of pregnancy, 198 ± 7.5 μm on the 18th day of pregnancy, and 374 ± 15.3 μm on the 21st day of pregnancy [Kawarabayashi et al.

1997]. After the administration of oxytocin in a dose of 200 nM, noticeable shortening of the length of the uterus muscular coat cells was observed. The length of individual cells amounts, respectively, to 97 ± 10.4 μm on the 15th day of pregnancy, w 130 ± 10.6 μm urn on the 18th day of pregnancy, and 164.8 ± 18.8 μm on the 21st day of pregnancy [Kawarabayashi et al. 1997]. The change of the length of the uterus muscular coat cells is also accompanied by a change to their width. On the 15th day of pregnancy, the width of smooth muscle fibres amounts to 13.2 ± 0.9 μm, on the 18th day of pregnancy – 14.5 ± 0.8 μm, and on the 21st day of pregnancy – 16.5 ± 1.0 μm, respectively [Kawarabayashi et al. 1997]. After the administration of oxytocin in a dose of 200 nM, the width of myocytes was subject to reduction up to a value of 12.4 ± 0.6 μm on the 15th day of pregnancy, 16.7 ± 1.0 μm on the 18th day of pregnancy, and 15.7 ± 1.0 μm on the 21st day of pregnancy, respectively [Kawarabayashi et al. 1997]. The study proves that contractions of the isolated longitudinal cells of the rat uterus muscular coat are the stronger, the more advanced their pregnancy is.

According to Kawarabayashi et al. [1997], oxytocin influences also a growth of intercellular calcium in the isolated myocytes of rat uterus smooth muscles. The administration of oxytocin in a dose of 10 nM results in a quick growth of calcium inside the isolated cells of the rat uterus muscular coat up to a value of 81 ± 11 nM on the 21st day of pregnancy. The growth occurred between 4th and 5th second from the administration of oxytocin. A drop in the intercellular calcium up to a value of 20 ± 9 nM took place after 20 seconds [Kawarabayashi et al. 1997]. On the 15th and 18th day of pregnancy, the growth of calcium inside the myocytes of rat uterus under the influence of oxytocin was not noted. A higher dose of oxytocin (200 nM) causes the growth of calcium inside the isolated myocytes already on the 15th and 18th day of pregnancy (respectively, 10.3 ± 1.7 nM/s and 11.5 ± 1.4 nM/s), however this growth is much lower than on the 21st day of pregnancy (30 ± 2.5 nM/s) [Kawarabayashi et al. 1997].

INFLUENCE OF DIFFERENT CONCENTRATIONS OF ACTIVE SUBSTANCES OF THE CALCIUM CHANNEL BLOCKERS ON THE CONTRACTION ACTIVITY

The conducted study has pointed out that administration of calcium antagonists into the incubation bath organ results in a decrease in both frequency and strength of the uterine contractions or total atony of the spontaneous uterine contraction activity. It depends on concentration of an active substance of the calcium channel blockers.

Nifedypine is a calcium antagonist with the strongest effect [Coruzzi and Poli 1987, Coruzzi et al. 1988, Subas et al. 2001). Administration of nifedypine in a dose of 2.8 x 10^{-8} mol/l into the incubation bath organ causes a drop in both strength and frequency of the contractions, whereas a ten-times higher dose (2.8 x 10^{-7} mol/l) leads to atony of the rat uterus muscular coat. In order to achieve a drop in strength and frequency of the uterine contractions with the use of diltiazem, a dose with a concentration of 0.66 x 10^{-4} mol/l is needed, whereas a ten-times higher dose (0.66 x 10^{-3} mol/l) leads to atony of the uterus muscular coat. Administration of verapamil in a dose of 0.5 x 10^{-4} mol/l into the incubation bath organ causes a drop in strength and frequency of the contractions, and a ten-times higher dose (0.5 x 10^{-3} mol/l) leads to atony of the rat uterus muscular coat. The conducted study proves that nifedypine in the concentration 10,000 times lower than diltiazem and verapamil causes blocking of the calcium channels, whereas diltiazem and verapamil show similar strength, which is proved by the study carried out by

Coruzzi et al. [1987 and 1988]. After the total blocking of the calcium channels with nifedypine, diltiazem and verapamil, and an occurrence of atony of the uterus smooth muscles, the administration of oxytocin in a dose of 0.01 i.u. into the incubation bath organ did not cause an increase in the contraction activity of an isolated strip.

According to Coruzzi et al. [1988] an inhibiting impact of the calcium channel blockers is not linked with an occurrence of phases of the rat oestrus cycle. Administration of nifedypine into the incubation bath organ both in the oestrus period (in a dose of 14.60 ± 3.3 nmol/1), in the dioestrus period (in a dose of 55.8 ± 16.1nmol/l) and in the pregnancy period (in a dose of 6.70 ± 2.8 nmol/1) caused atony of the spontaneous uterus muscular coat contraction activity. Verapamil and diltiazem caused the same effect, however, in much higher concentrations of the active substances: respectively, 1.53 ± 0.87 μmol/1 and 1.56 ± 0.80 μmol/1 in the oestrus period, 1.89 ± 0.72 μmol/l and 1.80 ± 0.86 μmol/1 in the dioestrus period, and 1.66 ± 0.8 μmol/1 and 0.47 ± 0.22 μmol/1 in the pregnancy period.

Subas et al. [2001] described the influence of nifedypine and diltiazem on the uterus muscular coat contraction activity in rats with hyperthyroidism and hypothyroidism. They proved that nifedypine is a drug that acts stronger on inhibiting of the spontaneous uterine contraction activity than diltiazem [Hollingsworth and Downing 1988]. According to Subas et al. [2001], both nifedypine and diltiazem have a weaker effect on a drop in the uterine contraction activity in rats with hypothyroidism than in rats with a proper level of hormones T3 and T4 in the animal's organism. Concentration of nifedypine necessary to achieve atony of the uterus muscular coat in rats with hypothyroidism amounts to 1 x 10^{-9} mol/l. In rats with a proper level of thyroid hormones, such a dose amounts to 1 x 10^{-6} mol/l. There is the analogous situation with diltiazem. Administration of this calcium channel blocker into the incubation bath organ in a dose of 1 x 10^{-5} mol/l results in atony of the uterus muscular coat in rats with T3 and T4 hormone hyposecretion, whereas in rats with a proper level of hormones T3 and T4 in the animal's organism, a dose of diltiazem that leads to atony of the spontaneous uterus muscular coat contraction activity amounts to 1 x 10^{-4} mol/l mol/l.

According to Coruzzi et al. [1988], inhibition of the uterus muscular coat contractility by the calcium channel blockers is more solid, longer lasting and more effective compared to inhibition of contraction activity through stimulation of β-adrenergic receptors with a selective agonist. The inhibiting effect on the uterus muscular coat can be used for the therapeutic purposes, however it can also have a negative effect on the reproductive system functioning [Czerski et al. 2004a], rarely cause of endometrial atrophy.

CONCLUSIONS

1. An isolated strip of rat uterus incubated in the automatic water bath organ shows spontaneous periodical contraction activity for about 8–12 hours.

2. Administration of oxytocin into the incubation bath organ causes an increase in frequency (by 44.85 ± 13.42%) and strength (by 39.5 ± 18.33%) of the spontaneous uterine contraction activity.

3. Administration of the calcium channel blockers into the incubation bath organ: nifedypine, diltiazem and verapamil causes a drop or atony of frequency and strength of

the uterus muscular coat contractions depending on concentration of an active substance of the preparation.

4. Nifedypine is a calcium channel blocker with the strongest effect. Blocking of the calcium channels with nifedypine in a dose of 2.8×10^{-8} mol/l caused a drop in frequency by 40 ± 9.86%, whereas a ten-times higher dose (2.8×10^{-7} mol/l) caused total atony of the spontaneous uterus muscular coat contractility.

5. Administration of oxytocin into the incubation bath organ after prior total blocking of the Ca^{2+} channels does not cause an increase in the contraction activity of the isolated strip.

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WPŁYW WYBRANYCH BLOKERÓW KANAŁÓW WAPNIOWYCH NA AKTYWNOŚĆ SKURCZOWĄ MACICY SZCZURÓW W WARUNKACH *IN VITRO*

Streszczenie. Badania przeprowadzono na 20 szczurzycach linii Bufflo w wieku 4–6 miesięcy, od których pośmiertnie pobrano skrawki macicy. Izolaty montowano w 4 komorach automatycznej łaźni wodnej. Po zapisaniu spontanicznej aktywności skurczowej wyizolowanego skrawka do komory podawano blokery kanałów wapniowych: diltiazem, nifedypinę, werapamil. Dawki preparatów ustalano doświadczalnie. W przeprowadzonych badaniach stwierdzono, że wyizolowany skrawek macicy wykazuje spontaniczną, cykliczną kurczliwość przez około 8–12 godzin. Średnia częstotliwość kurczliwości wynosiła 1,36 ± 0,33 skurczu na minutę, a średnia siła skurczu kształtowała się na poziomie 2,7 ± 0,7 grama. Podanie do komory inkubacyjnej oksytocyny powoduje wzrost częstotliwości (o 44,85 ± 13,42%) i siły (o 39,5 ± 18,33%) spontanicznej aktywności skurczowej macicy. Podanie do komory inkubacyjnej blokerów kanałów wapniowych: nifedypiny, diltiazemu i werapamilu powoduje spadek lub zanik (atonię) częstotliwości i siły skurczów mięśniówki macicy w zależności od koncentracji substancji czynnej preparatu. Najsilniej działającym blokerem kanałów wapniowych jest nifedypina. Zablokowanie kanałów wapniowych nifedypiną w dawce $2{,}8 \times 10^{-8}$ mol/l powodowało spadek częstotliwości o 40 ± 9,86%, natomiast dawka 10-krotnie wyższa ($2{,}8 \times 10^{-7}$) powodowała całkowity zanik spontanicznej kurczliwości mięśniówki macicy. Podanie do komory inkubacyjnej oksytocyny po uprzednim całkowitym zablokowaniu kanałów Ca^{2+} nie powoduje wzrostu aktywności skurczowej wyizolowanego skrawka.

Słowa kluczowe: kanały wapniowe, nifedypina, diltiazem, werapamil, kurczliwość macicy, szczur

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