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The histochemical characteristic of pancreatic B-cells

It is known that zinc possesses important role in processes of synthesis and formation of the deposited form of in pancreatic B-cells. Pancreas of many animals as of human contains a large amount of zinc. It is known also that some of chemicals and drugs capable to interact with zinc in B-cells that result destruction and death of cells. Authors have investigated the content of zinc in pancreatic islets of rabbit and features of localization in B-cells. It is established that the main amount of zinc concentrates in B-cells adjoining walls of blood capillaries through which there is hormone exit in blood. Authors suppose that destruction of pancreatic islets past intravenous injection of diabetogenic chemicals result for the first of all — destruction of B-cells adjoining to blood capillaries in islets.

Key words: B-cells, pancreas, zinc, insulin.

It is known a important role of zinc on processes of biosynthesis and storage of insulin in B-cells as deposited form in the ratio 1:6 of many animals and human [1,2]. A large amount of zinc is revealed in a pancreas and his prevalence in pancreatic islets is established [3]. Zinc takes part in metabolism and ensuring effect of hormones of hypo- physis, adrenal glands, pancreas and prostate [4]. The pancreas, hypophysis, eye retina, prostate and salivary gland contains a large amount of zinc [5]. It wassupposed that when crystal insulin is released from β-cells, his crystals are dissolved and hexamer dissociates on active monomers of insulin and ions of Zn^{2+} [4,6]. It was confirmed a important role of zinc in the pathogenesis of experimental diabetes induced by chemicals formed in B-cells toxic complexes with zinc that result destruction and death of cells within short period [7].

Aim of work: 1) to reveal zinc ions in B-cells of pancreas of Rats and Rabbits using specific and sensitive histochemical methods; 2) to investigate ability of zinc of B-cells to interact with diabetogenic chemicals.

Material and methods

In experiences pancreas tissue of adult 12 Rabbits were used. A water-ammonium of Dithizon solution (DZ) («SERVA», Germany) 46–49 mg/kg and ethanol solution of 8PTSQ (Institute of Pure Reagents, IREA, Moscow, Russia), 35–39 mg/kg were inje- cted to Rabbits.

DZ as 8PTSQ, a diabetogenic chelat active chemicals possess ability to form color red and green complexes with zinc visible in microscope [8]. Fixation of pancreas tissue in Bouin for 24h and at temperature of 0–5 °C in 70°etanol saturated with hydrogen sulfide for staining of zinc in paraffin sections of pancreas.

Frozen section of fresh pancreas tissue were investigated using dark microscopy and for staining of paraffin sections of fixed pancreas 4 methods were used: acetone solution of 8PTSQ for histochemical staining of zinc ions in B-cells [9], aldehyde-fucshine method [10] as immunohistochemical and

diethylpseudoisocyanine methods for staining of insulin [(11]. Intensity of staining and of fluorescence of B-cells were measured using of histofluorimetric complex [12,13]. To control intact animals equi- valent volumes of physiological solution was injected. Fixation of pancreas at temperature of 0…-5 °C in 70°etanol saturated with hydrogen sulfide.

8-para (toluenelsulphonilamino) quinoline (8PTSQ)- possess ability to form chelat complexes with Zn^{+2} ions as 1:1. This method of histochemical identification of Zn ions is high specific and very sensitive, allowing to reveal very low concentrations of Zn ions correspond to 10^{-7}–10^{-8} [14]. In ultra-violet light — wavelength equal of 360–370 nm — the Zn-8PTSQ complex fluoresces as brightly green light complex [8]. Meanwhile it is necessary to note that these chelat active chemicals possesses high chemical affinity to Zn ions and in the conditions of in vitro formed color chelat complexes Zn-8PTSQ visible at luminescent microscopy and a complex as Zn-Dithizon (DZ) visible as bright red granules using microscopy in the dark field.

For histochemical fluorescent staining of Zn ions 0,04 % aceton solution of 8PTSQ was used: 3–4 drops of solution placed on sections of tissues for 8–10 sec. following was- hing in the distilled water. Then sections were investigated using fluorescent microscopy

Preparing of Ditizon solution. For preparation of solution of Ditizon: 30 ml of the distilled water, 0,6 ml of 25 % of solution of ammonia and 400 mg of Ditizon were placed in vessel. Solution was mixed on a water bath (+70 °C) within 10 min., filtered using of ashless filter. The filtrate contains approxima- tely 1 % water-ammoniac solution of Ditizon which we used in our researches. Cytochemical indicators measuring of Zinc ions content were estimated as conventional units [12,13] (c.u.).

Results

Intravenous administration to rabbits and to mice of Ditizon and of 8PTSQ 5 min. later result formation of specific complexes Zn^{+2}- DZ and Zn-8PTSQ visible on frozen sections in B-cells contains a large amount of zinc (Figures 1.3; 1.5) comparatively with negative reaction for zinc in B-cells of animals with experimental diabetes (Figures 1.2; 1.6). Same result we have received past staining of zinc ions on paraffin sections of fixed tissue of pancreas. Using of luminescent and light microscopy the intensity of fluorescence and luminescence were measured on frozen sections past staining by 8PTSQ and by Dithizon in compared with control. The results showed about absence of any significant differences between indicators of intensity of fluorescence in B-cells past staining by 8PTSQ as of luminescence past staining by Dithizon (1,94±0,12 and 1,88±0,06 c.u.; 1,98±0,10 and 1,92±0,07 c.u.) respectively.

Results of staining of insulin showed that decrease in amount of zinc in B-cells was accom- panied by decrease in amount of insulin (Figures 1.1; 1.2; 1.7–1.10) and was confirmed by histofluorimetric method using of immunohistochemical and pseudoi-socyanine methods (1,91±0,04 and 1,07±0,03 c.u.; 2,05±0,05 and 1,12±0,04 c.u.) respectively.

Analysis of results investigation of insulin and zinc localization in B-cells shown that maximal amount of insulin as of zinc are localized on the pole of B-cells which contact to blood capillaries in B-cells (Figures 1.1; 1.3). Results of measure of density of staining of B-cells located around capillaries is evidently more high in compared with intensity of cells located in other part (Figures 1.1–1.4) of islets: insulin (2,64±0,18 and 1,46±0,04 c.u.) and zinc (2,76±0,22 and 1,35±0,08 c.u.). For measuring we have used aldehyde-fucshine staining and frozen sections of pancreas past injection of Dithizon; magnification as 15x40. Formation of complex zinc-helator in B-cells located around capillaries past injection of small doses of diabetogenic zincbinding chelators or at animals with experimental diabetes was especially distinctly observed. The complex zinc-ditizon located in the form of rings around capillaries (Figure 1.4) repeating his borders. Results of measuring of insulin and zinc content demonstrated that concen- tration as of zinc as insulin almost 2 times more high in pole of B-cells which contact with capillaries wall (Table, Figure 1).

Table

Localization of insulin and zinc in pancreatic B-cells

N	Animals	Insulin and zinc content in B-cells (c.u.)					
		Totally in B-cells of hole islet		Part of B-cells contacted capillaries (islet's central part)		Part of B-cells not contacted capillaries (islet's central part)	
		Insulin	Zinc	Insulin	Zinc	Insulin	Zinc
1	Intact	1,92±0,07 [1,2,3]	1,98±0,11	2,64±0,18 [1,4]	2,76±0,22	1,46±0,04 [2,4]	1,35±0,08
2	Diabetes	1,08±0,04 [3]	1,02±0,01	1,31±0,24	1,29±0,16	1,03±0,02	1,02±0,02

1,2,3,4 — $p<0,005$

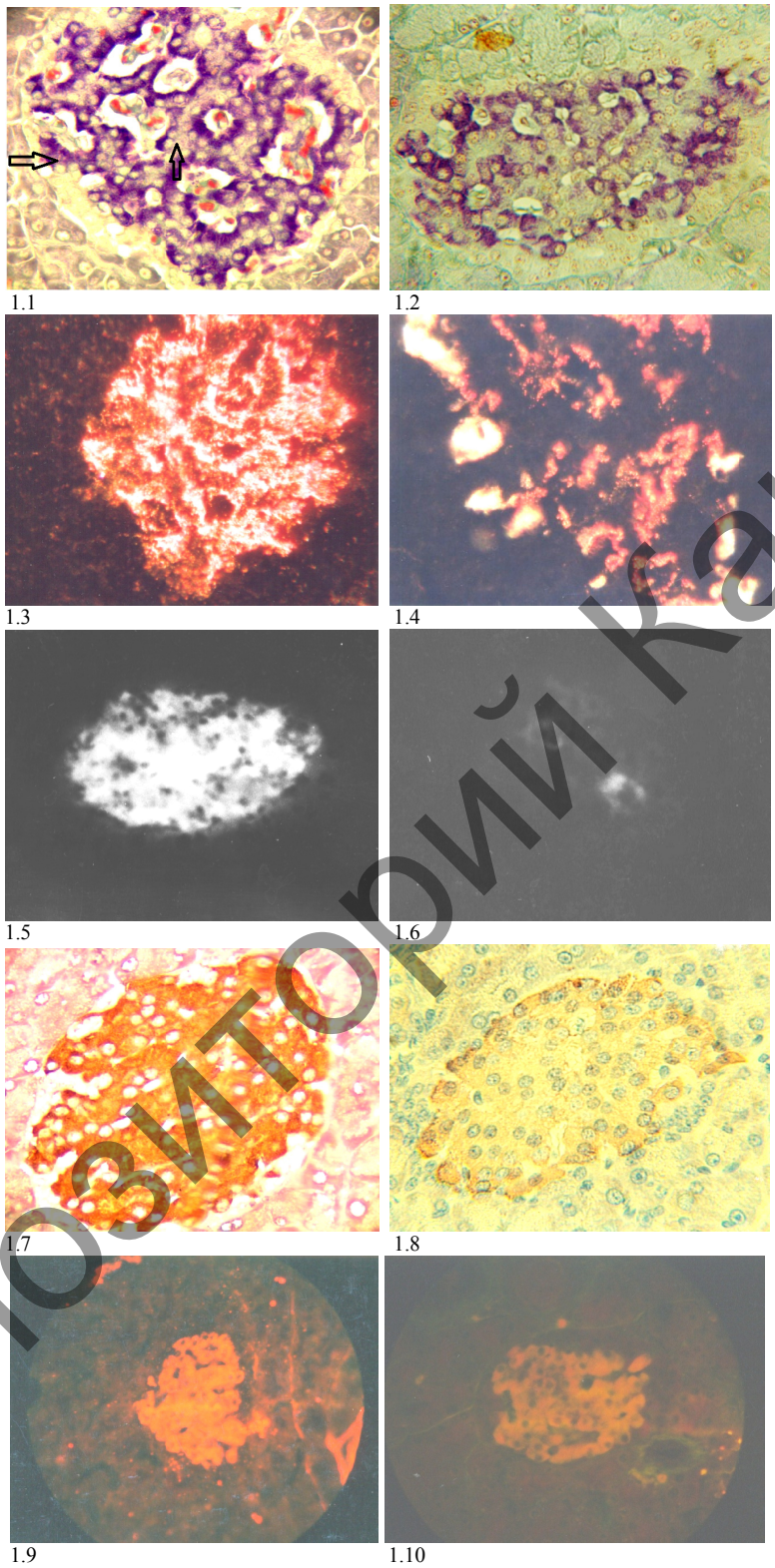

Figure 1. Insulin and Zinc in B-cells of pancreas

1.1. Islet of intact Rabbit. Aldehyde-fucshine. Maximal concentration of Insulin (violet color) in B-cells contacted capillaries wall; x280; 1.2. Islet of diabetic Rabbit. Aldehyde-fucshine. Negative reaction for insulin; x280; 1.3. Islet of Rabbit past injection of Dithizon, 46,3 mg/kg. Maximal concentration of Zinc (red color) in B-cells contacted capillaries wall; x280; 1.4. Islet of Rabbit past injection of Dithizon, 46,3 mg/kg. Negative reaction for Zinc; x280; 1.5. Islet of intact Rabbit. Zinc positive reaction with 8PTSQ; x120; 1.6. Islet of diabetic Rabbit. Negative reaction for Zinc with 8PTSQ; x120; 1.7. Islet of intact Rabbit. Immunohis tochemical staining. Positive reaction for insulin; x280; 1.8. Islet of diabetic Rabbit. Immunohistochemical staining. Negative reaction for insulin; x280; 1.9. Islet of intact Rabbit. Pseudoisocyanine staining. Positive reaction for insulin (red fluorescence); x120; 1.10 Islet of intact Rabbit. Pseudoisocyanine staining. Decreasing of insulin content in B-cells; x120

Results show also that aldehyde-fucshine and dithizon methods of staining are more suitable for studying of character localization of zinc and insulin in B-cells. Insulin and zinc formed color violet and red granules with paraldehyde and with dithizon. Concentration of granules in various parts of pancreatic islets allows to estimate more pre- cisely nature of localization of insulin and zinc in islets as to measure density of staining. Fluorescent histochemical technics and Victoria-4 method result homogeneous staining of zinc and insulin and complicates studying of features of localization as of zinc as hormone in islets. Besides, fluorescence partially extends to the B-cells located nearby that complicates analysis.

On the base of obtained results we suppose that this part of B-cells contacted capillaries wall past intravenous injection of diabetogenic zincbinding chemicals in blood first of all can be destroyed due to 2 reasons: 1) this B-cells first of all contacts to diabetogenic zincbinding chemicals delivered with blood as cells have direct contact to capillaries; 2) on this pole of B-cells the maximal amount of zinc is collects.

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Ұйқы безінің В-жасушаларының гистохимиялық сипаттамасы

Мақалада ұйқы безінің В-жасушаларында депо күйдегі инсулин синтезі және қалыптасуында мырыштың атқаратын рөлі аса маңызды екені белгілі және ол көмірсу алмасуын қамтамасыз етеді. Мырыш көптеген жануарлар мен адамның В-жасушаларында аздаған мөлшерде кездеседі. Кейбір дәрілік препараттардың құрамына кіретін химиялық заттар қатары В-жасушаларындағы мырышпен әрекеттесуге бейім, нәтижесінде олардың бұзылуына және диабеттің туындауына әкеледі. Авторлардың зерттеуінде панкреатит аралшықтарындағы мырыш мөлшері және В-жасушаларындағы шоғырлану ерекшелігі көрсетілген. Мырыштың көптеген мөлшері инсулинмен кешенді түрде В-жасушаларында жинақталатыны және қан капиллярларының қабырғаға жанаса орналасып, қанға гормонның бөлінуін қамтамасыз ететіні аталып көрсетілген. Авторлар химиялық диабетогендік заттардың қан арқылы өтуінде аралшықтардың жойылуы бірінші кезекте аралшық қабырғаларындағы қан капиллярларына жанаса орналасқан В-жасушаларының бұзылуынан басталатындығын дәлелдеген.

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Гистохимическая характеристика В-клеток поджелудочной железы

Цинку принадлежит важная роль в процессах синтеза и формирования депонированной формы инсулина в В-клетках поджелудочной железы, благодаря чему обеспечивается регуляция углеводного обмена. Цинк содержится в значительных количествах в В-клетках многих животных и человека. Ряд химических веществ, в том числе являющихся компонентами некоторых лекарственных препаратов, способны соединяться с цинком в В-клетках, приводя к их разрушению и развитию диабета. Авторами исследованы содержание цинка в панкреатических островках и особенности локализации в В-клетках. Установлено, что наибольшее количество цинка в виде комплекса с инсулином накапливается в В-клетках, примыкающих к стенкам кровеносных капилляров, через которые происходит выход гормона в кровь. Авторы полагают, что разрушение островков при попадании химических диабетогенных веществ через кровь начинается в первую очередь с разрушения В-клеток, примыкающих к стенке кровеносных капилляров в островках.

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