THE EFFECT OF HYPERGLYCEMIA ON THE ACTIVATION OF PERITONEAL MACROPHAGES OF ALBINO RATS

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Hyperglycemia is one of the main damaging factors of diabetes mellitus (DM). The severity of this disease is most clearly manifested under conditions of the inflammatory process. In this work, we have studied the activation features of rat peritoneal macrophages (MPs) under conditions of high glucose concentration *in vitro*. Comparison of the independent and combined effects of streptozotocin-induced DM and hyperglycemia on proliferation and accumulation of nitrites in the MPs culture medium revealed similarity of their effects. Elevated glucose levels and, to a lesser extent, DM decreased basal proliferation and NO production by MPs *in vitro*. The use of the protein kinase C (PKC) activator, phorbol ester (PMA), abolished the proinflammatory effect of thrombin on MPs. This suggests the involvement of PKC in the effects of the protease. At the same time, the effect of thrombin on the level of nitrites in the culture medium demonstrates a pronounced dose-dependence, which was not recognized during evaluation of proliferation. Proinflammatory activation of MPs is potentiated by hyperglycemia, one of the main pathological factors of diabetes. Despite the fact that high concentrations of glucose have a significant effect on proliferation and NO production, no statistically significant differences were found between the responses of MPs obtained from healthy animals and from animals with streptozotocin-induced DM. This ratio was observed for all parameters studied in the work, during analysis of cell proliferation and measurement of nitrites in the culture medium. Thus, the results obtained indicate the leading role of elevated glucose levels in the regulation of MPs activation, which is comparable to the effect of DM and even "masks" it.

Key words: macrophages; hyperglycemia; thrombin; streptozotocin-induced diabetes mellitus; nitric oxide; proliferation

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INTRODUCTION

According to the International Diabetes Federation (IDF), 537 million people with diabetes mellitus (DM) were registered in the world in of 2021 and their number is increasing every year. DM is characterized by a persistent increase in blood glucose levels, hyperglycemia, which acts as the most significant pathological factor of this disease. Hyperglycemia contributes to vascular damage, which leads to the development of diabetic complications such as nephropathy, neuropathy, angiopathy, retinopathy, diabetic foot, etc. [1, 2]. Pronounced hyperglycemia indicates the severity of the disease. DM, as well as its complications, is linked with the occurrence and development of inflammatory processes. An important role in inflammatory reactions is played by macrophages (MPs), immune cells with microbicidal and antitumor activity; their differentiation is determined by stimuli from the surrounding tissue. Over the past decade, extensive research has focused specifically on the role of MPs as key players in inflammation [3, 4]. MPs are characterized by plasticity and are able to quickly adapt to a wide range of biological signals [5]. The secretory activity of MPs can be both pro-inflammatory and anti-inflammatory in nature [6]. It is known that proinflammatory cytokines secreted by adipose tissue MPs impair insulin signaling in adipocytes [5]. The resulting insulin resistance contributes to the development of hyperglycemia, which leads to increased formation of advanced glycation end products and stimulates the production of reactive oxygen species by MPs and endothelial cells [8, 9]. In addition, these persistent inflammatory stimuli significantly reduce the wound healing effect of MPs [10–12]. As a result, foot ulcers are a leading cause of amputation in diabetic patients.

However, according to currently existing alternative viewpoint, that immune cells are involved in the maintenance of tissue integrity and the accumulation of MPs may be a protective mechanism aimed at combating metabolic stress [13]. Thus, the mechanism of activation of MPs and their role in pathological processes are not completely clear.

MP activity is regulated by membrane receptors, including protease-activated receptors (PAR), responding to their agonist, the serine protease thrombin [14, 15]. It was shown that activation of PAR by thrombin

on microglial cells led to an increase in the expression of inducible NO synthase (iNOS), the production of nitric oxide (NO), and activation of the transcription factor NF-κB [16]. The use of selective protein kinase C (PKC) inhibitors under these conditions reduced the thrombin effect on cell activation. Interestingly, activation of PKCβ also occurs in hyperglycemia and DM [17], and it is considered as one of the main links in pathophysiological changes in DM [18]. It has been shown that inhibition of PKCβ activity in diabetic conditions delays the development of diabetic complications, and in some cases improves conditions of diabetic patients [17].

PKC activation and its involvement in diabetic complications are also associated with other mechanisms, such as oxidative stress and mitochondrial dysfunction, which play a key role in hyperglycemic vascular damage. The vascular changes caused by PKC activation are mediated by VGSF, TGFβ1, endothelin 1 and other proteins that impaired microcirculation [19].

Currently, the effect of high glucose levels on the activation of MPs, as well as their activation by serine proteases of hemostasis, is not clear. In this regard, the goal of our study was to investigate the mechanisms of activation of rat peritoneal MPs under conditions of normo- and hyperglycemia.

MATERIALS AND METHODS

Research Object

The experiments were performed on a primary culture of MPs isolated from the peritoneal cavity of 3–4-month-old male Wistar rats weighing about 300 g. There were 9 animals in the control group and 5 animals in the diabetic group. The animals were kept in the vivarium of the Department of Human and Animal Physiology at 23°C, the standard daily light/dark cycle (12 h / 12 h), dry food and water for animals — *ad libitum*.

Induction of Experimental Diabetes Mellitus

Experimental type 1 DM was induced by a single intraperitoneal injection of streptozotocin (Sigma, USA) at a dose of 40 mg/kg. Streptozotocin is a substance that damages pancreatic β-cells and thereby reduces insulin production in the body, which is typical for type 1 DM [20]. This method of DM induction is used in many studies [21, 22]. A streptozotocin solution (30 mg/ml) was prepared in 0.1 M citrate buffer (pH 4.2) immediately before administration. Control animals were injected with citrate buffer. Blood glucose levels were measured one week after streptozotocin administration using a glucometer (OK Biotech Co. Ltd, Taiwan). An animal was considered as if the blood glucose level exceeded 16.66 mM (300 mg/dl).

Isolation of Peritoneal Macrophages

Peritoneal MPs were obtained according to the protocol described previously [23]. Control and diabetic animals were anesthetized with chloroform, decapitated, and phosphate-buffered saline (PBS; 10 ml) (Amresco, USA) was injected into the peritoneal cavity. The resulting wash from the peritoneal cavity was centrifuged for 5 min at 300 *g*. Further manipulations with cells were carried out under sterile conditions using a BMB-II-Laminar-S-1.3 NEOTERIC (Lamsystems, Germany). The resulting sediment was resuspended in DMEM medium (PanEco, Russia) containing 0.5 mM L-glutamine, 10% inactivated fetal bovine serum (HI-FBS), 100 units/ml penicillin/streptomycin (Gibco, USA), and 1 g/l (5.5 mM) glucose. Cell counting and viability assessment were carried out using a TC20 cell counter (Bio-Rad, USA) after preliminary staining of the suspension with the Trypan Blue dye. A cell suspension (2.5×10^5 live cells per well) was placed on 48-well culture plates at 37°C, 5% CO_2. After 2 h, the medium was completely changed to remove unattached cells. Using this protocol it is possible to obtain a MP culture with a purity of more than 90% [23].

Activation of Peritoneal Macrophages

The resulting MPs were incubated for 24 h in the presence of 10 nM or 50 nM thrombin (Sigma), 100 ng/ml lipopolysaccharide (LPS, Lipopolysaccharides from *Escherichia coli* O111:B4, L3024, Sigma), 100 nM phorbol ester (PMA, Phorbol 12-myristate 13-acetate, Sigma), 100 nM calcium ionophore A23187 (A23, Sigma). The MP activators used were diluted to the required concentration in a medium (DMEM) with normal (5.5 mM) or high (25 mM) glucose concentration and added to cell cultures. Previously, in a similar model, it was shown that the high concentration of glucose increased MP proliferation [24].

The degree of MP activation was assessed by proliferation and NO production.

Assessment of Peritoneal Macrophage Proliferation

Analysis of MP proliferation was carried out using the WST-1 test in accordance with the manufacturer's recommendations. After 24 h-incubation of MPs with the test substances, the culture medium was replaced with a fresh one containing 10% WST-1 (Sigma) and incubated for 40 min. Then the optical density of the solution was measured at 450 nm using an iMark microplate absorbance reader (Bio-Rad).

Assessment of Nitrite Accumulation in Peritoneal Macrophage Culture In Vitro Using Griess Reagent

The level of NO production was assessed by the concentration of nitrites in the medium, according to a previously described protocol [25].

Briefly, 50 µl of medium from the cell supernatant collected 24 h after the addition of activators was mixed with 50 µl of Griess reagent (Sigma). After 30 min of incubation, the optical density was measured using an iMark microplate absorbance reader (Bio-Rad) and a 530 nm filter.

Statistical Data Processing

Statistical analysis was performed using the GraphPad Prism 6.0 program (GraphPad Software, USA). Statistical data processing was carried out using one-way analysis of variance (one-way ANOVA) with Dunnett's correction for multiple comparisons. The data were checked for compliance with the normal distribution using the Shapiro-Wilk test. Differences were considered significant at $p<0.05$. The number n in each group is the number of independent experiments. Each group had at least 6 independent replicates. The data in the graphs were normalized relative to the control group at the corresponding glucose level (Fig. 1–4) or relative to the effect of the corresponding intervention at the glucose level in the medium of 5.5 mM (Fig. 5, 6). Data are presented as the mean ± standard deviation.

RESULTS AND DISCUSSION

The Effect of Glucose Levels on Pro-Inflammatory Activation of Macrophages In Vitro

The induction of streptozotocin-induced DM was accompanied by a persistent increase in the level of blood glucose. A week after the administration of streptozotocin to animals, the concentration of blood glucose reached an average of 26 mM, while in animals in the control group this parameter did not change, remaining at the level of 5.8 mM. In addition to a significant increase in blood glucose concentration, polydipsia, polyuria, and weight loss were observed in diabetic animals. Thus, the used model of DM is adequate and is accompanied by symptoms characteristic of this disease.

The effect of glucose levels on macrophage proliferation *in vitro* under the influence of proinflammatory stimuli

It is known that DM is accompanied by the maintenance of high blood glucose and a persistent, pronounced inflammatory response even to weak stimuli, which can lead to organ dysfunction and tissue necrosis. At the site of inflammation, not only activation of immunocompetent cells and their death are observed, but also proliferation [26].

In this regard, in the first series of experiments we have investigated the MP proliferation under stimulation conditions at normal (5.5 mM) and high (25 mM) glucose levels in the culture medium. It was found that the substances we used had different effects on cell proliferation. For example, at normal glucose levels, increasing concentrations of thrombin (10 nM and 50 nM), as well as LPS (100 ng/ml), increased MP proliferation compared to the control (without exposure to the substances) (Fig. 1A). Since PMA is often used to activate cells *in vitro* as a PKC activator, as well as increasing cytosolic calcium concentrations (for example, by means of the ionophore A23187), we have analyzed the effect of these substances on MPs at different glucose concentrations in the culture medium.

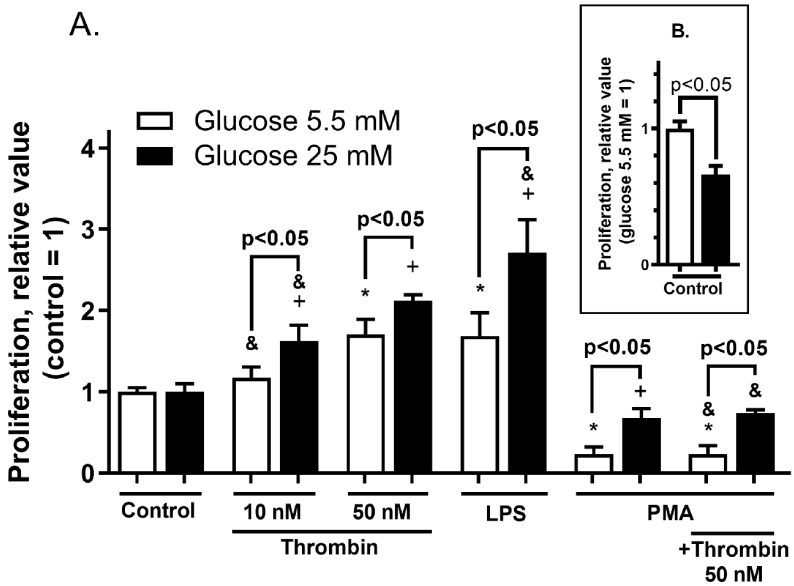

Figure 1. The effect of activators on the proliferation of MPs at normal (5.5 mM) and high (25 mM) glucose in the medium. **A)** Proliferation of MPs in the presence of activators. Data were normalized to the control (without activators) at the same glucose level. **B)** Proliferation of MPs in the medium without activators. Data were normalized relative to control at 5.5 mM glucose. LPS – lipopolysaccharide, PMA – phorbol ester. * – $p<0.05$ as compared to control at 5.5 mM glucose; + – $p<0.05$ as compared to control at 25 mM glucose; & – $p<0.05$ as compared to 50 nM thrombin at the same glucose concentration.

The addition of PMA to the macrophage culture medium at normal glucose concentration (5.5 mM) led to a decrease in cell proliferation; this effect was observed during PMA addition alone and in combination with 50 nM thrombin as compared to the control group (Fig. 1A). The addition of calcium ionophore A23187 to the MP culture medium had no effect on proliferation (data not shown).

Cultivation of MPs for 24 h in a medium containing high glucose (25 mM) led to almost 2-fold decrease in the number of cells as compared to the level of MP proliferation under similar influences, but when cells were cultured in a medium containing 5.5 mM glucose (Fig. 1B). This is consistent with the information available in the literature on the toxic effects of high concentrations of glucose [1]. Cultivation of peritoneal MPs under conditions of high glucose concentration (25 mM) for 24 h led to a significant increase in proliferation in response to the addition of all substances used, as compared with their effects at a normal glucose level in the medium (Fig. 1A). At the same time, thrombin and LPS increased the proliferation of MPs versus the control by 2.1 and 2.7 times, respectively. The observed effect of LPS differs significantly from the effect of thrombin on MP proliferation under these conditions. Such difference may indicate a modulating effect of high glucose concentration on the process of reception and intracellular signal transmission under the influence of thrombin and LPS; this was previously shown for thrombin receptors on endothelial cells and platelets, and for TLR4 on the myocardium [27–29]. Under high-glucose conditions, the effect of PMA on MP proliferation was higher than under normal glucose conditions; however, these proliferation values remained lower than those in the control group of cells (Fig. 1A).

Thus, an increase in glucose concentration in the MP culture medium *in vitro* potentiated the multidirectional effects on MP proliferation of LPS and thrombin on the one hand and PMA on the other.

The effect of glucose level on nitrite content in MP culture medium under the influence of various proinflammatory stimuli

Activation of MPs can be caused by different factors and can be accompanied by the secretion of both pro-inflammatory and anti-inflammatory mediators. For example, the bactericidal properties of MP M1 are determined by production of free radicals of nitrogen and oxygen, involving iNOS and the NADPH oxidase complex. Recently, it has been shown that increased NO production plays a significant role in the pathogenesis of DM [8, 11, 30]. Therefore, in the next series, we have analyzed the NO production MPs evaluated by the accumulation of nitrites in the cultivation medium.

Under normal glucose concentration (5.5 mM), activation of MPs by 10 nM and 50 nM thrombin and LPS was accompanied by an increase in NO production by 2.5, 7.2, and 12.5 times, respectively (Fig. 2A). Figure 2A shows that nitrite levels increased sharply when cells were incubated with 50 nM thrombin. This underlies the concentration dependence of the thrombin effect. Interestingly, the effect of thrombin on MP proliferation did not have such a pronounced dose-dependent manner (Fig. 1A). The effect of PMA on NO production was unidirectional with its previously observed effect on MP proliferation. Pretreatment of cells with PMA at normal glucose concentration (5.5 mM) abolished the effect of 50 nM thrombin on nitrite levels (Fig. 2A). The calcium ionophore A23187 did not affect the production of NO by MPs at normal glucose concentrations (data not shown).

The effect of 25 mM glucose on NO production by MPs was unidirectional with its effect on proliferation (Fig. 2B). At the same time, the effect of thrombin on the level of nitrite accumulation in the cell culture medium was similar during MP cultivation both in a medium with normal (5.5 mM) and high (25 mM) glucose. However, the LPS effect on the release of NO by MPs was different in cell culture medium with 5.5 mM glucose and with 25 mM glucose (Fig. 2A). When the glucose concentration in the medium increased from 5.5 mM to 25 mM, the effect of the combined action of PMA and thrombin (50 nM) significantly increased. This may indicate the dominating influence of thrombin on this parameter, as opposed to the effect of PMA.

Thus, we have found that LPS and thrombin (50 mM) exhibit a unidirectional proinflammatory effect on MPs and cause an increase in NO production and MP proliferation. Cultivation of MPs in a medium with high glucose concentrations potentiated the effect of these activators on cell proliferation. The independent effect of high glucose was characterized by both inhibition of proliferation and a decrease in NO release by peritoneal MPs *in vitro*. This decrease in NO release under conditions of high glucose may be associated both with a decrease in NO synthase activity, which is observed in hyperglycemia associated with DM, and with depletion of the L-arginine pool available for NO synthase [31, 32].

PKC activation under these conditions was similar to the effect of high glucose concentrations on the studied parameters. Moreover, an increase in glucose content in the MP culture medium reduced the differences between changes in nitrate accumulation and cell proliferation caused by the action of PMA from the control parameters. This may indicate a similar mechanism of action of PMA and hyperglycemia on the studied parameters. At the same time, activation of PKC abolished thrombin-induced increase in both proliferation and, to a lesser extent, NO release. This effect is likely due to the inhibitory effect of PMA on the PKC functioning. Certain evidence exists in the literature on the similar effect of long-term incubation of cells in the presence

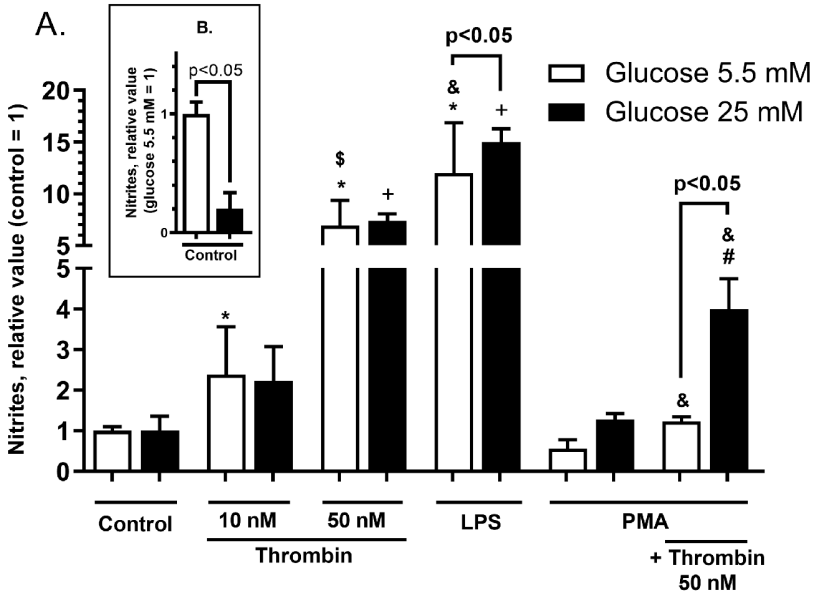

Figure 2. The influence of activators on the production of nitric oxide (NO) by MPs in the medium with normal (5.5 mM) and high (25 mM) glucose concentrations. NO production was assessed using the Griess reaction by the concentration of stable metabolites (nitrites). **A)** Effects of activators on NO concentration in the culture medium. Data were normalized to the control (without activators) at the same glucose level. **B)** NO concentration in the cultivation medium without activators. Data were normalized to the control at 5.5 mM glucose. LPS – lipopolysaccharide, PMA – phorbol ester. * – $p<0.05$ as compared to control at glucose 5.5 mM; + – $p<0.05$ as compared to control at glucose 25 mM; $ – $p<0.05$ compared to thrombin 10 nM at the same glucose concentration; & – $p<0.05$ as compared to thrombin 50 nM at the same glucose concentration; # – $p<0.05$ as compared to PMA at 25 mM glucose.

of high PMA concentrations [33]. Since PKC mediates the pro-inflammatory effects of thrombin, its inhibition should lead to a decrease in the thrombin effect, which is confirmed by our data.

The Effect of Streptozotocin Induced DM on Proinflammatory Activation of Macrophages In Vitro

It is known that DM is accompanied by changes in the state of immunocompetent cells; this probably determines the characteristics of the development of inflammation in this disease. To study the activation of MPs under diabetic conditions, we have used the generally accepted model of streptozotocin-induced DM in rats. Peritoneal cells were collected on day 7 after streptozotocin administration, blood glucose was above 16.6 mM. In DM, there was a decrease in both cell proliferation and nitrite accumulation, but this effect was less pronounced than the effect of a high glucose concentration on MPs (see previous section, Figs. 3B, 4B). An assessment of the proliferation of MPs isolated from diabetic rats showed an increase in this parameter only in the presence of LPS and 50 nM thrombin, but not PMA and 10 nM thrombin (Fig. 3A). At the same time, in contrast to the effect of a high glucose concentration, DM did not increase, and in the case of application of 50 nM thrombin even decreased this parameter as compared to the effects observed on control MPs (from non-diabetic animals). The effect of PMA on the proliferation of MPs in diabetic rats was similar to its effect on MPs under conditions of high glucose concentration in the medium (Fig. 1A, 3A). Measurement of nitrite levels confirmed the unidirectional effect of DM on the specific action of activators on the proliferation of MPs when exposed to 25 mM glucose.

The decrease in the effects of activators (with the exception of PMA) on diabetic MPs found by us in comparison with the effect of high glucose levels, may be determined by the altered state of cells exposed to hyperglycemia under diabetic conditions *in vivo* and then treated *in vitro* by the activators used. As a result, the responsiveness to *in vitro* stimulation of MPs obtained from diabetic animals reduced as compared to 24-h exposure to the high glucose.

The Effect of Glucose Levels in the Culture Medium on Pro-Inflammatory Activation of Macrophages from Rats with Streptozotocin-Induced Diabetes Mellitus

In the next series of experiments, we performed a comparative analysis of the effect of high glucose on activator-induced responses of MPs from control and diabetic animals. The change in the action of activators under the influence of high glucose was similar in the case of MPs obtained from control animals (without diabetes) and those obtained from diabetic animals (Figs. 5, 6).

For example, the high glucose promoted a significant decrease in the proliferation of MPs isolated from nondiabetic control animals and exposed to 50 nM thrombin (Fig. 5). It is possible that reactions induced by high glucose, for example, production of reactive oxygen species, can lead to cell death and/or

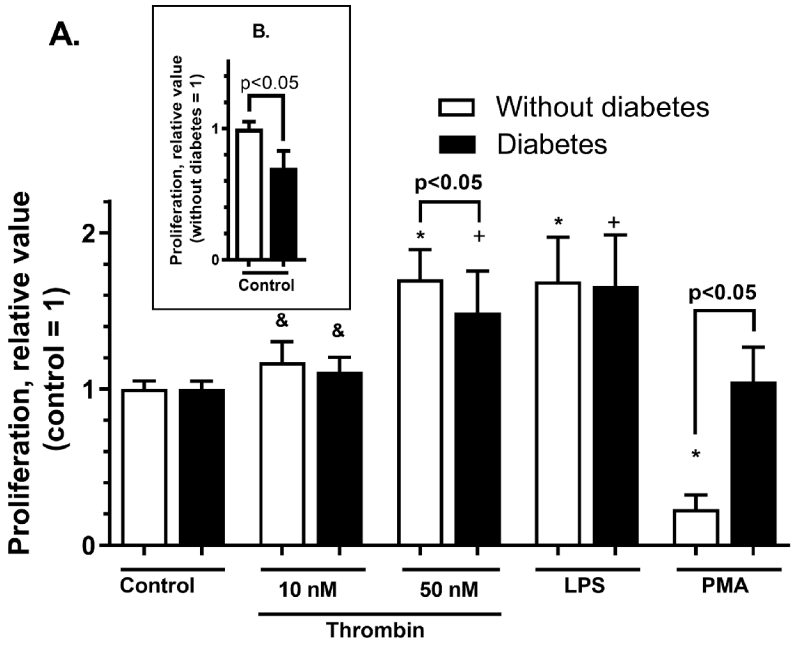

Figure 3. The effect of activators on the proliferation of MPs isolated from healthy and diabetic rats. **A**) Proliferation of MPs in the presence of activators. Data were normalized to the corresponding MPs without activators. **B**) Proliferation of MPs in the medium without activators. Data were normalized to the control group without diabetes. LPS – lipopolysaccharide, PMA – phorbol ester. * – $p<0.05$ as compared to the control (without DM); + – $p<0.05$ as compared to the diabetic control; & – as $p<0.05$ compared to 50 nM thrombin at the same exposure (MPs from healthy or diabetic animals).

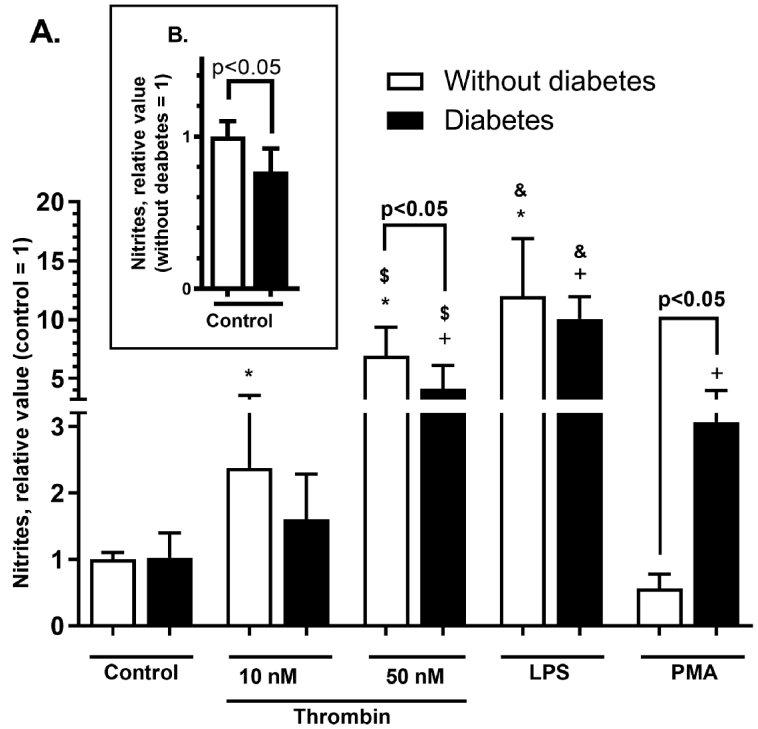

Figure 4. The influence of activators on the NO production by MPs isolated from healthy and diabetic animals. NO production was assessed using the Griess reaction by the concentration of stable metabolites (nitrites). **A)** Effects of activators on the NO concentration in the MP culture medium. Data were normalized to the control MPs (without activators) from corresponding animals (with or without DM). **B)** NO concentration in the MP cultivation medium without activators. Data were normalized relative to the control group without diabetes. LPS – lipopolysaccharide, PMA – phorbol ester. * – $p<0.05$ as compared to the diabetic control; + – $p<0.05$ as compared to the non-diabetic control; $ – $p<0.05$ as compared to the effects of 10 nM thrombin on MPs from the same group (with or without DM); & – $p<0.05$ as compared to the effect of 50 nM thrombin on MPs from the same group (with or without DM).

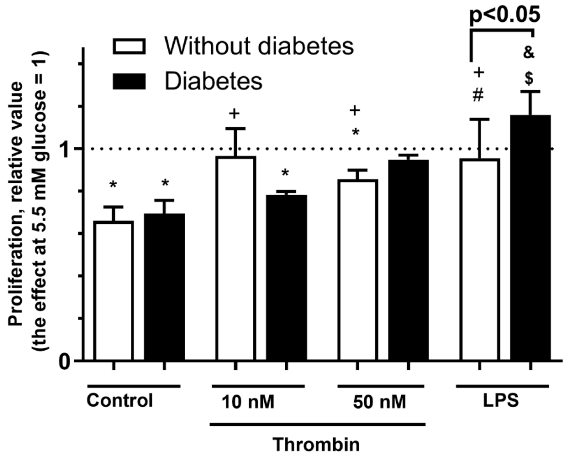

Figure 5. The effects of activators (or their absence) on the proliferation of MPs (from control and diabetic rats) during their cultivation under conditions of high glucose. Data were normalized to the effect of the corresponding activator (or its absence) in MPs (with or without DM) at normal glucose (5.5 mM) (defined as 1). LPS – lipopolysaccharide. * – $p<0.05$ compared with the effect of exposure at glucose 5.5 mM (defined as 1); + – $p<0.05$ compared to control in the absence of diabetes; $ – $p<0.05$ compared with diabetic control; & – $p<0.05$ compared with the effect of 10 nM thrombin on diabetic MPs.

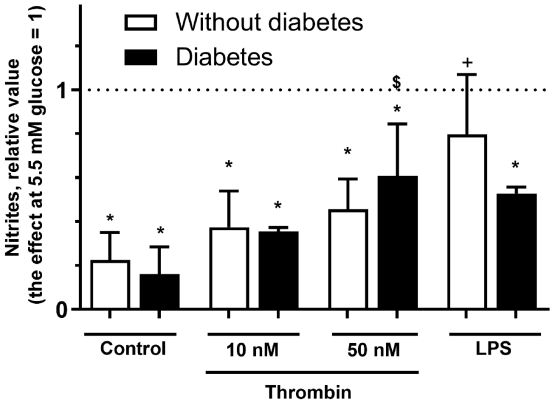

Figure 6. The effects of activators or their absence on the NO production by MPs isolated from control or diabetic animals and cultivated in the medium with high glucose. NO production was assessed using the Griess reaction by the concentration of stable metabolites (nitrites). Data were normalized to the effect of the corresponding activator (or its absence) in MPs (with or without DM) at normal glucose (5.5 mM (defined as 1)). LPS – lipopolysaccharide. * – $p<0.05$ as compared with the effect of exposure at 5.5 mM glucose (defined as 1); # – $p<0.05$ as compared with PMA, 50 nM thrombin in the absence of diabetes; + – $p<0.05$ compared to control in the absence of diabetes; $ – $p<0.05$ as compared to the diabetic.

a decrease in their resistance to pathogens and toxic factors. For example, a decrease in proliferation in the presence of 50 mM thrombin may indicate an increase in the MP sensitivity to the toxic effect of protease in the presence of 25 mM glucose (Fig. 5). It was previously shown that high concentrations of thrombin could cause cell death [34]. Similar changes in proliferation of MPs obtained from diabetic animals were found, as in the previous case, in the group without activators and in MPs treated with 10 nM thrombin. Thus, the absence of significant differences in the relative changes in proliferation caused by the factors used under conditions of hyperglycemia (as compared to the corresponding effects under conditions of normoglycemia) between the control group and the group of diabetic animals indicates a "masking" of the effects caused by increased glucose levels.

The only significant difference in MP proliferation was found between the MPs isolated from control and diabetic animals and exposed to LPS. Moreover, the LPS effect on MPs from diabetic animals led to an increase in its effect with increasing glucose levels in the medium. At the same time, proliferation of MPs isolated from control animals under conditions of high glucose in the medium did not differ from the value of this parameter in normoglycemia (5.5 mM) (Fig. 5).

Analysis of nitrites in the culture medium showed that at high glucose, there was a decrease in NO production by MPs, regardless of their origin (i.e. from control or diabetic rats) (Fig. 6). MPs obtained from diabetic animals demonstrated a decrease in NO production at high glucose both in the absence (control group) and in the presence of activators (10 nM and 50 nM thrombin, LPS) (Fig. 6). A similar result was obtained for cells from control animals (without DM), with the exception of MPs treated with LPS, where the LPS effect at high glucose did not differ from that at normoglycemia (Fig. 6).

CONCLUSIONS

High glucose concentration has a significant impact on the proliferation and NO production by MPs *in vitro*. Moreover, an increase in glucose to 25 mM in the culture medium neutralized the differences in MP responses to proinflammatory stimuli between control and DM groups of animals. This ratio was observed for all parameters studied in the work, both in the analysis of cell proliferation and in the measurement of nitrites in the culture medium.

Thus, the results obtained indicate the leading role of elevated glucose levels in the regulation of MP activation, which is comparable to the effect of DM and even "masks" it.

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COMPLIANCE WITH ETHICAL STANDARDS

All experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ВЛИЯНИЕ ГИПЕРГЛИКЕМИИ НА АКТИВАЦИЮ ПЕРИТОНЕАЛЬНЫХ МАКРОФАГОВ БЕЛЫХ КРЫС

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Гипергликемия является одним из основных повреждающих факторов сахарного диабета (СД). Тяжесть данного заболевания наиболее ярко проявляется на фоне воспалительного процесса. В данной работе исследовали особенности активации перитонеальных макрофагов (МФ) крысы в условиях высокой концентрации глюкозы *in vitro*. В ходе оценки пролиферации и накопления нитритов в среде культивирования МФ и при сравнении изолированного и сочетанного влияния стрептозотоцинового сахарного диабета (СД) и гипергликемии установлено сходство эффектов СД и гипергликемии на МФ. Показано, что повышенный уровень глюкозы и в меньшей степени СД снижают базовую пролиферацию и продукцию NO МФ *in vitro*. Использование активатора протеинкиназы С (ПКС) форболового эфира (ФЭ) отменяло провоспалительное действие тромбина на МФ, что может свидетельствовать о вовлечении ПКС в эффекты протеазы. При этом, влияние тромбина на уровень нитритов в среде культивирования демонстрирует выраженный дозозависимый характер, что не выявлено при измерении пролиферации. Провоспалительная активация МФ потенцируется гипергликемией — одним из основных патологических факторов СД. Несмотря на то, что глюкоза в высокой концентрации оказывает существенное влияние на пролиферацию и продукцию NO, не было выявлено статистически значимых различий между ответами МФ, полученных от здоровых животных, и МФ от животных со стрептозотоциновым СД. Такое соотношение наблюдалось по всем исследуемым в работе параметрам и при анализе пролиферации клеток, и при измерении нитритов в среде культивирования. Таким образом, полученные результаты указывают на ведущую роль повышенного уровня глюкозы в регуляции активации МФ, которая сопоставима с эффектом СД и даже “маскирует” его.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: макрофаги; гипергликемия; тромбин; стрептозотоцин-вызванный диабет; оксид азота; пролиферация

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