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Research Article

INSULIN EFFECTS ON HUMAN PLEURAL ELECTROPHYSIOLOGY: INSIGHTS FROM COMPARATIVE STUDY WITH PLEURA AND INTERVENTION WITH GLIBENCLAMIDE

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ABSTRACT

The study aimed to investigate the direct effect of insulin on human pleural electrophysiology, analogous to findings observed in sheep pleura. Utilizing glibenclamide, a hypoglycemic agent, as a glucoward, researchers sought to determine if insulin elicited similar effects in human pleura, the involvement of insulin receptors, and the potential reversibility of these effects by glibenclamide. Human parietal pleural specimens were mounted in a Ussing chamber, and various solutions containing glibenclamide, insulin with anti-insulin antibody, and insulin with anti-insulin receptor antibody were applied. Transmesothelial resistance (RTM) was measured using the RTM method, while immunohistochemistry was employed to detect insulin receptors (IRa, IRb). The study found that anti-insulin and anti-insulin receptor antibodies inhibited insulin-induced changes in RTM when applied mesothelially within the first minute. Moreover, glibenclamide effectively eliminated insulin-induced alterations. Immunohistochemistry revealed evidence of IRb and IRa. These findings suggest that interference with insulin receptors leads to electrochemical changes in both humans and sheep pleura, which can be mitigated by glibenclamide intervention.

Keywords:- Diabetes, Glibenclamide, Insulin, Immunity.

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INTRODUCTION

A major mediator of anabolism and glucose control, insulin plays an important role. The insulin oedema syndrome is a rare but potentially dangerous complication in diabetic patients who receive insulin as treatment. Typically, it is characterized by oedema and fluid accumulation throughout the body, ranging from simple ankle oedema to severe cardiopulmonary manifestations like pulmonary oedema, cardiogenic shock, or pleural effusions [1–4]. The permeability of epithelia, especially those in the distal kidney, has been hypothesized to be altered by insulin in insulin oedema, causing electrolyte retention and fluid accumulation resulting from insulin oedema [5–7]. In diabetic patients who develop insulin oedema syndrome, pleural effusions remain unexplained despite the aforementioned explanation. A direct effect of insulin on sheep's pleura was previously demonstrated in an effort to explain this event. . Specifically, insulin blocked ion transporters that have been implicated in pleural fluid recycling, such as amiloride-sensitive Na+ channels and ouabain-sensitive Na+/K+ pumps. Pleural trans-mesothelial resistance increased as a result of these electrochemical changes. [8, 9] An insulin-insulin receptor interaction was hypothesized to be responsible for the aforementioned

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effect based on the presence of insulin receptors a (IRa) and b (IRb) in sheep pleura. A similar effect of insulin on the electrochemical profile of the human parietal pleura was examined during this study, along with whether its receptor interacts with it, and whether glibenclamide reverses this effect (also used for diabetes treatment).

MATERIALS AND METHODS

A total of forty-four, intact pleuras were collected from patients having thoracic surgery (through thoracotomies and thoracoscopic procedures) for treatment of lung cancer during the course of the study. Specimens dissected from the lung mass were not adjacent to each other. Histopathological examination of each specimen was conducted, and the histology report confirmed that none of the specimens had any disease. Exclusion criteria included people with pleural effusion prior to surgery, abnormal blood sugar levels preoperatively, or diabetic history. In 30 minutes after tissue dissection, the remaining specimens were cooled to 4°C in preoxygenated Krebs solution. All patients participating in the study gave their consent after the study was approved by the local ethics committee.

In contrast, interstitial surface refers to the surface of the pleura facing the chest wall, whereas mesothelial surface faces the pleural cavity. Using-type chambers were used to mount the pleural tissues as planar sheets. In order to maintain tissue viability, both sides of the tissue were immersed in Krebs solution, which was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide gas at 37°C. Three (3) tissue sections were dried overnight at 60 degrees Celsius on slight amounts of human parietal pleura. Using a microwave oven (LG WAVEDOM 850 Watt), slides were deparaffinized with xylene and rehydrated with decreasing levels of ethanol. A blocking solution of 0.3% hydrogen peroxide was incubated for 10 minutes on the sections after they had been cooled, washed with PBS, and soaked in 0.3% hydrogen peroxide. Each section was then blocked for 1 hour after washing with PBS. An incubation period of 30 minutes followed the addition of envision fluid. A counterstain of hematoxylin was applied to the slides and they were mounted. Positive controls were pancreatic and liver tissue sections. A positive control was considered to be the blood vessel wall of the mesothelium specimens. Negative controls were obtained by omitting the incubation process with the primary antibodies.

RESULTS

Addition of insulin on the mesothelial surface increased RTM rapidly, within the 1st minute (from 20.99 ± 0.5Ω·cm^2 to 22.85 ± 0.6 Ω·cm^2, dRTM 1.86 Ω·cm2, versus control P = .016). This effect lasted for 5 min (22.11 ± 0.6 Ω·cm2, versus control, P = .026), and RTM was decreased thereafter till baseline (20.92 ± 0.6 Ω·cm2, versus control, P>.05) after 30 minutes. Little effect was observed interstitially (20.99 ± 0.5 Ω·cm2 to 21.47 ± 0.6 Ω·cm2, dRTM 0.48 Ω·cm2, versus control P>.05). 3.2. Effect of Anti-Insulin Antibody on Insulin-Induced Alterations. The anti-insulin antibody totally inhibited the insulin-induced effect (from 22.85 ± 0.6 Ω·cm2 to 21.05 ± 0.6 Ω·cm2 P = .01, versus control P>.05). The anti-insulin receptor antagonist also totally inhibited insulin-induced effect (from 22.85 ± 0.6 Ω·cm2 to 20.95 ± 0.6 Ω·cm2 P = .01, versus control P>.05.

Effect of Anti-Insulin-Like Growth Factor 1 (IGF) Receptor Antibody on Insulin-Induced Alterations. The anti-IGF-1 receptor antagonist did not inhibit the insulin-induced effect (from 22.85 ± 0.6 Ω·cm2 to 22.54 ± 0.5 Ω·cm2P>.05, versus control P = .014.

Detection of IRa and IRb in Human Parietal Pleura. Mesothelial cells showed positive immunostaining for IRa and IRb. The immunoreactivity was cytoplasmic. The distribution of immunoreactivity was diffuse. Staining intensity was even and convincing.

DISCUSSION

As a result of adding insulin to the mesothelial surface of human parietal pleura, electrochemical changes are observed. Insulin elicits this effect by interacting with its receptors found in the parietal pleura of humans according to immunohistochemistry. Human parietal pleura electrochemical profiles were weakly affected by another commonly used hypoglycaemic agent, glibenclamide, which reversed insulin-induced effects. In other tissues, insulin increases short circuit current primarily towards the mesothelial side of the tissue within the first five minutes [11]. PDTM of alveolar type II cells was increased when insulin was added mesothelly [12, 13].

Diffusion-induced interstitial effects of insulin were noted in toad bladders [11]. This study found weak interstitial effects owing to diffusion [14, 15] or residual fat or blood clots [16]. When insulin is added apically to kidney cells, it stimulates amiloride-sensitive Na+ channels. Based on previous findings [17], we used this concentration of insulin in this study. The lack of pleural effusions during insulin therapy may be explained by the high insulin concentrations rarely reaching such high levels in insulin-treated humans. It is possible that insulin diffuses into the pleural cavity following diabetes treatment depending on bloodstream levels. In pleura, insulin reduces permeability rather than augments it, like in kidneys. This high concentration could also explain why insulin decreases permeability in pleura.

The kidneys were even observed to produce interstitial effects when insulin concentrations used in high concentrations were applied to the epithelia of their epithelia [18]. Insulin enhances glucose uptake by

interfering with its receptor, as has been demonstrated numerous times. Aside from interfering with Na+ transport in epithelial tissues, insulin also interacts with its receptors [19, 20]. Insulin also regulates permeability in colonic cells via receptor-mediated mechanisms [21]. The basolateral side of human bronchial epithelial cells displays a greater concentration of insulin receptors [22]. Observed electrochemical alterations in the human parietal pleura are attributed to insulin and its interactions with insulin receptors a (IRa) and b (IRb).

Based on the results mentioned above, insulin stimulated a similar electrochemical response in sheep and humans. As well as insulin receptors a (IRa) and b (IRb), both species contain insulin receptors. Therefore, sheep and human pleura present many similarities electrophysiologically and histopathologically, indicating that sheep can be an acceptable animal model for extrapolating findings to humans. Because consent is required, it is challenging to obtain human tissue, and its stripping causes bleeding in healthy subjects. Also, IGF-1 increases transcellular ion flux across epithelial membranes, and when it interacts with its receptor, it changes the kidney's permeability. It is possible that insulin interacts with IGF-1 receptors because insulin and IGF-1 receptors have similar structures. The inhibition of the insulin receptor would not have inhibited insulin's action in pleura because the IGF-1 receptor was free to bind with insulin, which indicates insulin binds to the IGF-1 receptor to cause its effect. Inhibition of the insulin receptor would have partially inhibited insulin's effect in the pleura if insulin binds to the insulin/IGF-1 receptor to initiate its action. In some specimens coadded with insulin and anti-IGF-1 receptor antibody, insulin's effect in the pleura at least is not dependent on the IGF1 receptor. The role of IGF-1 receptor in insulin action in pleura, however, needs to be clarified through further research.

Inhibition of adenosine triphosphate-sensitive K+ channels (K(ATP)) is one way that glibenclamide improves insulin production by pancreatic cells. As an agent that interferes with electrolyte transport in kidneys, glibenclamide interferes with these channels. A possible harmful effect of this drug is its involvement in mitochondrial K(ATP) function. Our results did not support such a harmful effect on electrophysiology. The effect of glibenclamide in the pleura was even more significant, as it inhibited the electrochemical response to insulin. It is plausible that insulin-insulin receptor binding is hindered as ATP is depleted due to the depletion of cellular ATP. As glibenclamide did not induce electrochemical or permeability changes in pleura, its blockage of K+ transport via K(ATP) channels needs to be clarified. The effects of glibenclamide on the kidney and myocardium were also selected as parts of its hypoglycemic role.

Based on the results of the present study, insulin presence in the pleural cavity may reduce the permeability of the pleural membrane, which may explain why insulin-treated diabetics develop pleural effusions. As a result, insulin may alter the electrophysiological profile and affect pleural recycling by altering its electrophysiological profile. Other hypoglycaemic agents, such as glibenclamide, can reverse the oedema effect. Clinicians who encounter this rare complication of pleural effusion formation during insulin therapy may find this observation, although at an experimental level, to be a useful alternative treatment option.

CONCLUSIONS

Accordingly, insulin induced electrophysiological alterations of the human parietal pleura, similar to those caused in sheep pleura, and this interaction was mediated by a receptor. This finding, as well as the presence of these receptors in human and sheep parietal pleura, is indicative of their similarity. Glubenclamide completely inhibited this effect in the human parietal pleura without causing intense electrophysiological alterations.

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