THE EFFECT OF VARIOUS (1→3)-β-D-GLUCANS ON THE BRONCHOCONSTRICTOR ACTIVITY OF METHACHOLINE ON GUINEA PIG AIRWAY SMOOTH MUSCLE - AN *IN VITRO* STUDY

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Abstract

(1→3)-β-D-glucans are a diverse family of glucose polymers. They have been shown to mediate effects on the respiratory system, including influx of inflammatory cells and release of inflammatory mediators. It is believed that these pharmacological activities are dependent on the molecular weight, degree of branching, tertiary structure and solubility of the glucan. In order to further define the effect of (1→3)-β-D-glucans on the respiratory system, this present study evaluates whether these agents can alter the bronchoconstrictor activity of methacholine on airway smooth muscle in the guinea pig. Perfused lung and immersed tracheal tissue preparations were exposed to various doses of methacholine in the prescence of a variety of (1→3)-β-D-glucans, and the resultant bronchoconstrictor activity was determined. Both insoluble and soluble (1→3)-β-D-glucans reduced the contractor activity of methacholine on the tracheal smooth muscle. However, in the perfused lung preparation, only the soluble (1→3)-β-D-glucans promoted a decrease in response to methacholine. These results imply that the (1→3)-β-D-glucans may act by different mechanisms in these two respiratory tissues.

Introduction

(1→3)-β-D-glucans are natural products found in the cell walls of plants, fungi, moulds and some bacteria. They are a diverse family of compounds that differ greatly in their ultrastructure. They are glucose polymers that all share a characteristic (1→3)-β-D backbone, branching of the polymer can occur generating a (1→6)-β side chain. The ultrastructure of the (1→3)-β-D-glucans presents in various forms, varying from random coils to single helices and stable triple helices.

The physical characterisation of (1→3)-β-D-glucans is important as it appears that such properties may influence their pharmacological activity. Thus, in studies of the effect of various glucans on markers of respiratory inflammation, it has been found that release of cytokines (Okazaki et al., 1995), influx of inflammatory cells (Fogelmark et al., 1992; Fogelmark et al., 1994; Milanowski, 1997), and production of reactive oxygen species (Okazaki et al., 1996) within the airways are dependent on the molecular weight, degree of branching, ultrastructure and solubility of the (1→3)-β-D-glucans.

Respiratory diseases, such as asthma and byssinosis are characterised by airway inflammation. However, another feature is a degree of airway hyper-responsiveness. The aim of the present study was to establish whether (1→3)-β-D-glucans had any influence on the bronchoconstrictor responsiveness of airway smooth muscle.

Materials

Methacholine hydrochloride, glucan from baker's yeast, curdulan and laminarin were all purchased from Sigma Chemicals.

Grifolan was a gift from Professor Ragnar Rylander, University of Gothenberg, Sweden.

The glucan from baker's yeast, and curdulan were suspended in distilled water at a concentration of 1mg/ml. The suspensions were sonicated, using an MSE Soniprep 150, for 5 minutes to aid solubility.

The laminarin was dissolved in distilled water to give a concentration of 1mg/ml.

The grifolan was dissolved in the minimum quantity of 0.3N sodium hydroxide (20mg grifolan in 6ml of 0.3N NaOH) and the kept on ice until use.

All of the above stock solutions were then diluted to the required concentrations with Krebs solution for use in the experiments.

Method

Male Dunkin and Hartley guinea pigs (200-550g) were killed by cervical dislocation and exsanguination. The ribcage was removed and the respiratory tract excised into warmed Krebs solution (NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 11.1 mM). The tissue was cleared of extraneous material and blood vessels. Then, at the bifurcation, the trachea was cut and the lungs were separated into right and left lobes

The experimental set up described by Young and Nicholls (1996) was employed. Each lung was attached to a cannula and secured with cotton. The lungs were then perfused with Krebs solution, via a Watson and Marlow peristaltic pump, at a rate of 5ml/min. Pressure changes of the perfusion fluid within the lung were measured by a Bill and Hartley pressure transducer attached to a Devices MX4 recorder.

The trachea was cut into two 4cm spirals using the method of Constantine (1965). Each spiral was suspended in a 50ml organ bath filled with Krebs solution. A tension of 1g was then applied to the tracheal spirals. The contractions of the

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trachea were measured by a Dynamometer UFI isometric transducer attached to the same Devices MX4 recorder.

For both the lungs and the trachea, the Krebs solution temperature was maintained at 37◦C by means of a Grant circulator, and the solution was oxygenated by passing gas ($95\%O_2$, $5\%CO_2$) through it.

One lung and one trachea half were used as time controls to monitor spontaneous changes in reactivity of the tissue over the course of the experiment. In order to eliminate any apparatus and/or biological bias, "cross over" experiments were undertaken. For the first experiment the right lung was used as the "test tissue", for the second experiment it was the left lung and so on.

The tissues were allowed to equilibrate for approximately 1 hour, then a dose-response relationship to methacholine was determined. In the case of the trachea a full dose-response curve was performed, However, with the lungs, four doses that spanned the range of a standard dose-response curve to methacholine were used (1,3,10 and 300 µg).

The lungs were then perfused with one of the (1→3)-β-D-glucans (either glucan from Baker's yeast (glucan (BY)), curdulan, or laminarin), for 1 hour. After exposure to the glucan the lungs were then reperfused with normal Krebs solution. Immediately and 1 hour after reintroducing the Krebs solution the dose-response relationship was repeated to assess any changes in reactivity.

The tracheal spirals were immersed in either one of the above (1→3)-β-D-glucans or the remaining glucan - grifolan. After 35 minutes, and still in the presence of the (1→3)-β-D-glucan, the dose-response curve to methacholine was repeated.

All results were analysed using a paired Students t-test, the result was taken as significant when $P<0.05$.

Results

Exposure to 50µg/ml glucan (BY), an insoluble particulate glucan, caused a significant decrease in the reactivity of both the lungs (Figure 1) and trachea (Figure 2). This concentration of glucan (BY) has previously been shown to be the most effective at producing this response (Jones and Nicholls, 1997). The hypo-responsive effect of glucan (BY) on both tissues was observed at all doses of methacholine, including that of the maximal response of the tissue. In the perfused lung, this reduced response was sustained over a 1 hour period.

Exposure to 50µg/ml Curdulan, another insoluble glucan, did not significantly alter the reactivity of either of the preparations to methacholine (data not shown). When the concentration of the glucan was increased to 100µg/ml, there was a significant decrease in the response of the trachea to methacholine (Figure 3). However, the maximal response of the tissue was not reduced. When the lungs were perfused with this higher concentration of curdulan unexpected results were observed. The smooth muscle was unable to relax back to baseline after administration of the higher doses of methacholine. The magnitude of the response to methacholine did not alter, but the contraction was sustained over a considerably greater time period.

When 50µg/ml laminarin, a soluble, lower molecular weight glucan, was employed there was no effect in either the lungs or the trachea (data not shown). After increasing the concentration to 100µg/ml there was a significant decrease in the reactivity of the tracheal preparation to methacholine, including that at the maximum doses of agonist. (Figure 4). However, 100µg/ml laminarin had no significant effect on the perfused lungs' response to methacholine, apart from the response to 300µg methacholine 1h after the laminarin exposure (Figure 5).

There were no significant changes in response of the trachea after exposure to 50µg/ml grifolan (a gel forming, soluble, higher molecular weight glucan) (Figure 6). Nevertheless, the results do indicate that there is a trend towards a decrease in response.

For each experiment a parallel time control experiment was undertaken and in all cases there was no significant alteration of the response of the tissues to methacholine over the experimental time course.

Discussion

It is evident that (1→3)-β-D-glucans do have some effect on the reactivity of guinea pig respiratory smooth muscle to methacholine. The insoluble, particulate glucans, in particular glucan isolated from baker's yeast, are capable of causing hypo-responsiveness in the lungs and trachea and this effect is highly dose-dependent.

In assessing the effects of these insoluble glucans, the perfused lung model may be limited. The 100µg/ml concentration of curdulan had to be abandoned in this set of experiments because 1h post exposure readings were not achievable. The sustained contractions observed may be due to the particle size of the glucan suspension and physical blockage of the airways by these particles. A possible extension of this work would be to ascertain whether curdulan retains its ability to cause hypo-responsiveness in the trachea after it has been rendered soluble by treatment with sodium hydroxide.

Laminarin, a soluble glucan, only seems to have an effect on the trachea. Again, it causes a hypo-responsive effect in a dose-dependent manner. This may suggest that laminarin has a similar mode of action in the trachea to the insoluble (1→3)-β-D-glucans.

Grifolan, solubilized in sodium hydroxide, might possibly cause a decrease in response of the trachea. However, further concentrations would need to be administered before this could be confirmed.

These results imply that (1→3)-β-D-glucans act by different mechanisms in the lungs and the trachea. Only the particulate glucans have any effect in the lungs, whereas the trachea is affected by both soluble and insoluble glucans. This could be due to the presence of other components, rather than just smooth muscle, in the perfused lung preparation. It may be that the (1→3)-β-D-glucans are affecting other cells, such as macrophages and vascular endothelial cells, in the lung. This could cause release of other mediators that in turn affect the preparations ability to respond to methacholine.

However, it must be considered that all (1→3)-β-D-glucans could promote a decrease in reactivity of any guinea pig airway smooth muscle. It may be that the effect is highly dose-dependent and as yet the correct concentrations have not been utilised in the perfused lung preparation.

Lipopolysaccharide (LPS) is another environmental agent that is implicated in certain respiratory diseases. Like (1→3)-β-D-glucans, it too is found in various organic dusts and can cause an inflammatory response in the airways (Sandström et al., 1992). However, when bronchoconstrictor activity is examined, LPS has an opposite effect to (1→3)-β-D-glucans. LPS is capable of promoting a marked hyper-responsive action to methacholine in guinea pig respiratory smooth muscle (Young and Nicholls, 1996). Exposure to (1→3)-β-D-glucans often goes hand in hand with exposure to LPS. Therefore, the possible interaction of these two agents and the potential influence it might have on respiratory smooth muscle needs to be investigated.

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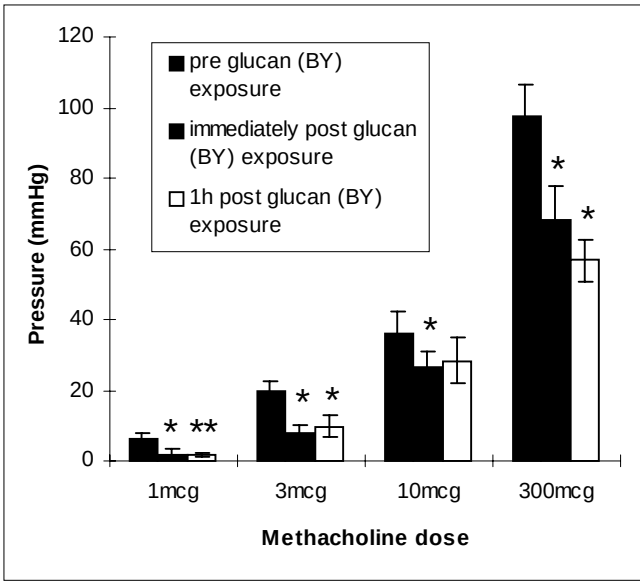

Figure 1: Change in reactivity to methacholine of a guinea pig perfused lung preparation after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * $P < 0.05$, ** $P = 0.05$

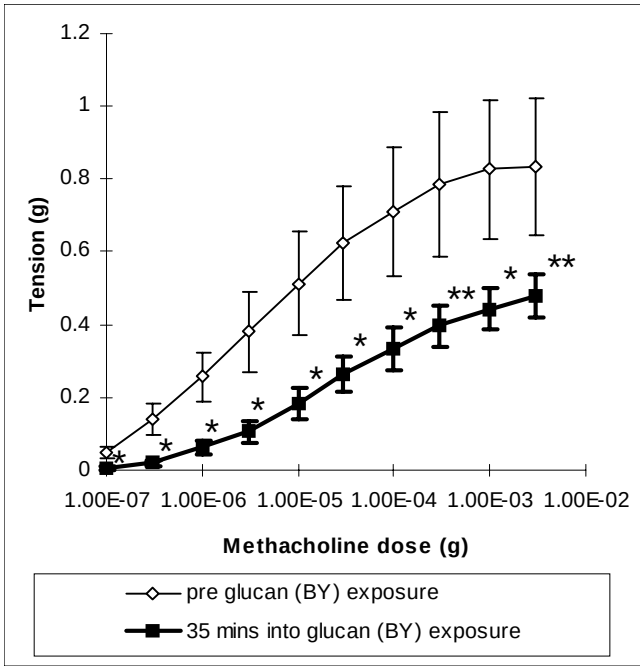

Figure 2: Change in reactivity to methacholine of guinea pig tracheal spirals after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

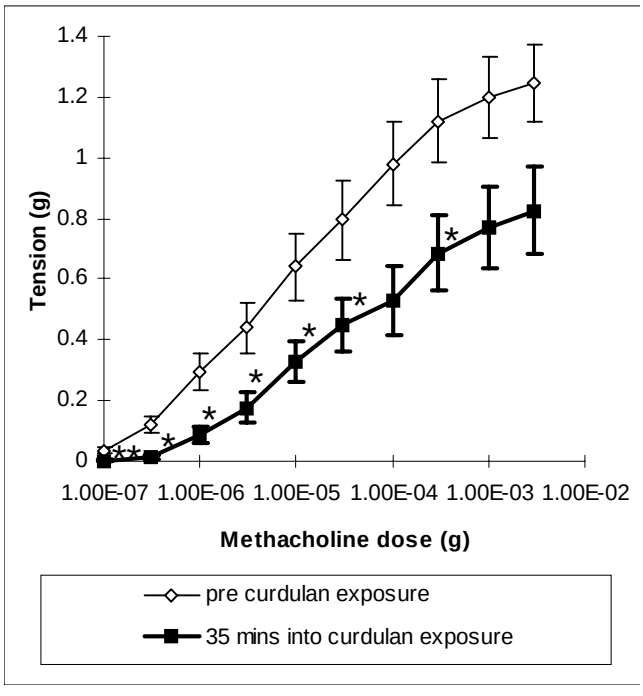

Figure 3: Change in reactivity to methacholine of guinea pig trachea spirals after exposure to curdulan 100µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

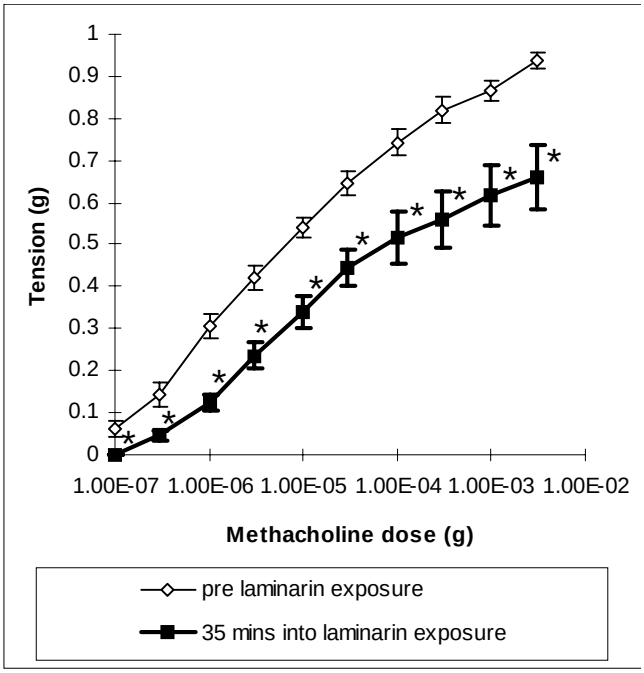

Figure 4: Change in reactivity to methacholine of guinea pig trachea spirals after exposure to laminarin 100µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

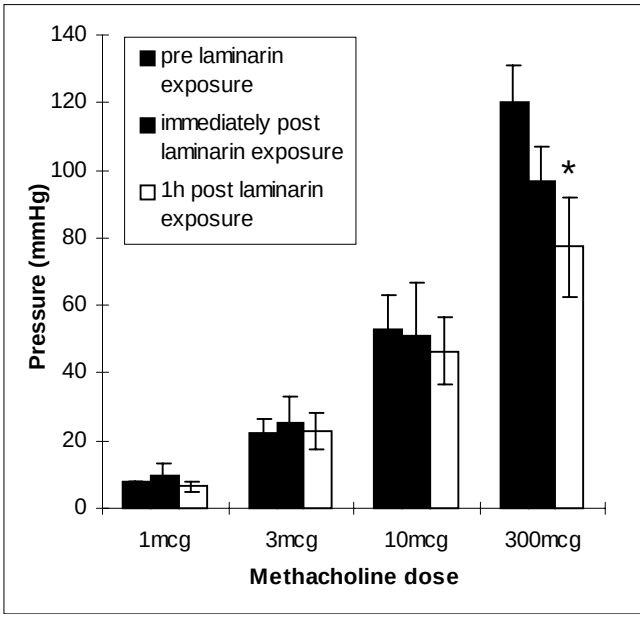

Figure 5: Change in reactivity to methacholine of a guinea pig perfused lung preparation after exposure to laminarin 100µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

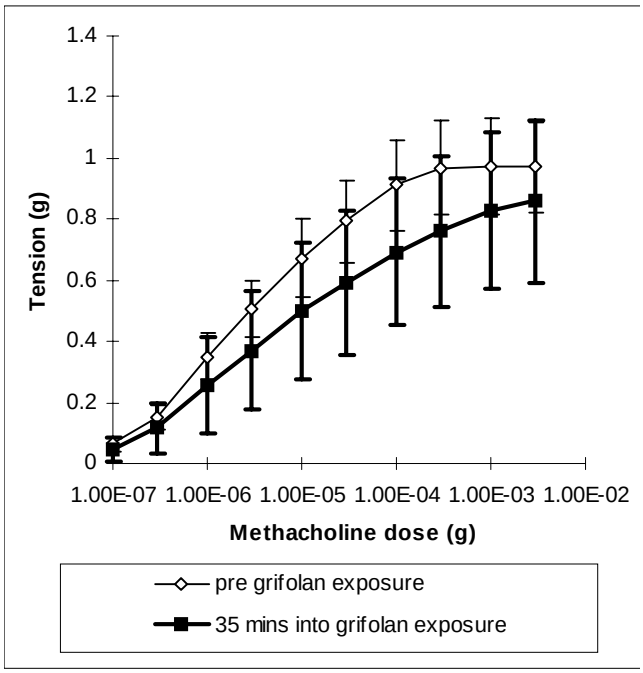

Figure 6: Change in reactivity to methacholine of guinea pig trachea spirals after exposure to grifolan 50µg/ml. Values are means ± s.e.m, n=6, * $P < 0.05$, ** $P = 0.05$