THE EFFECT OF (1→3)-β-D-GLUCAN ON THE ARACHIDONIC ACID CASCADE IN GUINEA PIG ISOLATED TRACHEA AND PERFUSED LUNG

A Jones and PJ Nicholls
Department of Pharmacology
Welsh School of Pharmacy
Cardiff University
Cardiff, UK

Abstract

It has been shown that glucan (isolated from baker's yeast) is capable of causing a decrease in reactivity of guinea pig airway smooth muscle (Jones and Nicholls, 1998). The present experiments were designed to help determine the mode of action of this (1→3)-β-D-glucan and the possible mediators involved. In particular, to ascertain if glucans have any effect on the arachidonic acid cascade. Using the immersed trachea and perfused lung models, various pharmacological tools were employed to try to modify the hypo-responsiveness seen after exposure to glucan. Antagonists of cyclo-oxygenase blocked the effect of the (1→3)-β-D-glucan on the trachea. However, in the lung, the results suggested that the 5-lipoxygenase pathway is probably of more importance. Overall, it appears that, this glucan may mediate some of its effects through the arachidonic acid cascade.

Introduction

It has previously been shown by our group that a (1→3)-β-D-glucan (isolated from baker's yeast) (glucan (BY)) is capable of causing hypo-responsiveness in the guinea pig respiratory system (Jones and Nicholls, 1998). Exposure to this particulate, insoluble glucan produces a decrease in reactivity to a variety of constrictors in the perfused lung and isolated trachea models.

Within the respiratory tract there is a plethora of mediators that may influence the contractility of respiratory smooth muscle. Some of these, at least, are involved in maintaining the inherent tone of the airways. Amongst these mediators are the arachidonic acid cascade products (Yamane and Kobayashi, 1990).

Arachidonic acid is released from membrane phospholipids by phospholipase A_2, and metabolised by two major pathways:

a) the lipoxygenase pathway - this produces both spasmogenic and pro-inflammatory leukotrienes.
b) the cyclo-oxygenase pathway - this produces a variety of prostaglandins, some of which can cause bronchodilation, and others which are involved in brochoconstriction and airway inflammation.

It is possible that exposure to glucan (BY) may influence the balance of arachidonic acid derivatives that are released in the tracheal and lung preparations. In order to investigate this, experiments were undertaken to look at the effect of various blockers of the arachidonic acid cascade on glucan (BY)-induced hypo-responsiveness. It was thus hoped to determine at what point, if any, this glucan affects the cascade.

Materials

Glucan (from baker's yeast), indomethacin, methacholine and phenidone were all obtained from Sigma Chemicals. Mepyramine maleate was purchased from Rhone Poulenc Rorer Ltd.

Glucan (from baker's yeast) was suspended in distilled water at a concentration of 1mg ml^{-1}. The suspension was sonicated, using an MSE Soniprep 150, for 5 minutes to aid solubility. This stock suspension was then diluted to the required concentration of 50µg ml^{1} at the time of use.

Mepyramine maleate was dissolved in distilled water then diluted to 1.0µM with Krebs solution.

Phenidone was dissolved in 0.9% NaCl, to give an orange solution, then diluted to 0.1mM with Krebs solution.

Indomethacin was dissolved in 0.1M Na_2CO_3 then diluted to 1.4µM with Krebs solution.

Method

Male Dunkin Hartley guinea pigs (200-500g) were killed by cervical dislocation and exsanguination. The respiratory tract was excised into warmed Krebs solution (NaCl 118, KCl 14.7, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 11.1mM). The trachea, and the right and left lungs, were then separated at the bifurcation.

Each lung was attached to a cannula and secured with cotton. One lung (control lung) was then perfused with Krebs solution. The other lung (test lung) was perfused with a pre-determined concentration of blocker in Krebs solution. The perfusion rate was set at 5ml min^{-1} using a Watson and Marlow peristaltic pump. Pressure changes of the perfusion fluid within the lung were measured by Bell and Hartley pressure transducers attached to a Devices MX4 recorder.

Using the method of Constantine (1965), the trachea was cut into two equal length spirals. Each spiral was then suspended, under a tension of 1g, in a 50ml organ bath. One spiral (control trachea) was immersed in Krebs

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:192-195 (1999)
National Cotton Council, Memphis TN

solution, the other tracheal spiral (test trachea) was immersed in pre-determined concentration of blocker in Krebs solution. The changes in tension of the tracheal spirals were measured by Dynamometer UFI isometric transducers attached to the same Devices MX4 recorder.

For all experiments, the Krebs solution was maintained at 37°C by means of a Grant circulator, and was oxygenated by passing 95% O_2 , 5% CO_2 gas through it.

The blockers used in these experiments were: mepyramine 1.0µM, phenidone 0.1mM and indomethacin 1.4µM.

After equilibration of approximately 1 hour, a dose response relationship to methacholine was determined in all tissues. In the tracheal spirals a full dose response curve was undertaken. However, in the lungs, four doses that spanned the range of a standard dose response curve to methacholine were selected (1,3,10 and 300µg).

The lungs, once the pressure had returned to baseline, were then perfused with 50µg ml^{-1} glucan (BY) for 1 hour. In the control lung, the glucan (BY) was diluted in pure Krebs solution, whereas in the test lung, the glucan (BY) was diluted in the blocker solution. After the hour period, the lungs were re-perfused with their original perfusion solutions. Immediately and 1 hour after re-introduction of these solutions, the dose response relationship to methacholine was repeated to assess any changes in reactivity.

With the tracheal spirals, once the tension had returned to its baseline reading, the control trachea was immersed in 50µg ml^{-1} glucan (BY) in Krebs. The test trachea was immersed in the same concentration of glucan (BY) but diluted in the blocker solution. After 35 minutes of the exposure, and still in the presence of glucan (BY), the dose response curve to methacholine was repeated.

All results were analysed using a paired Student's t-test, the result was taken as significant when $P<0.05$.

Results

All the control preparations (lung and trachea) showed the expected significant decrease in response to methacholine after exposure to 50µg ml^{-1} glucan (BY) (Figures 1 and 2). The reduction in sensitivity was observed at nearly all doses of methacholine, including that of the maximal response of the trachea.

The presence of 1.0µM mepyramine had no effect on the decrease in reactivity of the lungs and trachea after exposure to glucan (BY). (Figures 3 and 4). Thus there was still a significant reduction in the response to methacholine in these tissues. In order to ascertain that the mepyramine was at an effective antihistamine concentration in the tissues, positive controls were undertaken. Mepyramine significantly blocked the action of histamine in the trachea and lungs ($P<0.05$), but had no effect on the response to methacholine ($P>0.05$). This confirms that an appropriate concentration of mepyramine had been employed.

In the trachea, both the presence of phenidone (Figure 5) and indomethacin (Figure 6) inhibited the decrease in reactivity to methacholine after glucan (BY) exposure. There was no significant differences between the dose response curve to methacholine pre-glucan (BY) exposure and 35min into glucan (BY) exposure. It appears that both agents can eliminate the action of glucan (BY) in the trachea.

In contrast, it was only phenidone that appeared to inhibit the action of glucan (BY) in the lung (Figure 7). While indomethacin had no effect at all (Figure 8). Thus there is still significant hypo-responsiveness in the indomethacin treated lung after exposure to glucan (BY).

Discussion

As mentioned above, and as seen in Figures 1 and 2, glucan (BY) is capable of causing a decrease in sensitivity to a variety of constrictors in the lung and trachea.

The results indicate that, in the trachea at least, the action of glucan (BY) can be prevented by certain blockers. Firstly a standard pharmacological blocking agent was employed. Mepyramine, a H_1 antagonist, is known to block the action of histamine in the respiratory system. However, in the present models, mepyramine does not block the action of glucan (BY) in either the lungs or the trachea. This implies that glucan (BY) does not alter the action of histamine on smooth muscle in these tissues.

Phenidone, originally designed as a 5-lipoxygenase inhibitor, inhibits the action of both the 5-lipoxygenase and cyclo-oxygenase enzymes in the arachidonic acid cascade (McMillan and Walker, 1992). It is capable of inhibiting the formation of both leukotrienes and prostaglandins in the respiratory tract. The results in Figure 5 show that phenidone blocks the action of glucan (BY) in the trachea. This suggests that glucan (BY) may affect the balance of arachidonic acid cascade derivatives.

In order to further narrow down the precise action of glucan (BY) in the trachea, phenidone was replaced by indomethacin (a specific cyclo-oxygenase inhibitor). As shown in Figure 6 indomethacin also inhibits the decreased sensitivity to methacholine in the trachea. This result reinforces the possibility that glucan (BY) affects the arachidonic acid cascade. It appears that the action of glucan (BY), in the trachea, is mediated at some point in the cyclo-oxygenase pathway, perhaps affecting the release of certain prostaglandins.

The results in the perfused lung model suggest that phenidone, but not indomethacin, blocks the hypo-responsive action of glucan (BY). This implies that this glucan affects the 5-lipoxygenase pathway in the lung. Nevertheless, to confirm this, experiments using a specific 5-lipoxygenase inhibitor need to be carried out.

However, it must be considered that the perfused lung model is far more complicated than the tracheal model. The presence of other components, rather than just smooth muscle, may influence the effect that is seen. Therefore a combination of events may produce the overall effect that is seen . It is likely that when the glucan (BY) enters the lung it is taken up by macrophages, which then could cause release of different mediators. Recent work by Ljungham et al. (1998) has shown that macrophages stimulated with a (1→3)-β-D-glucan can stimulate formation and release of nitric oxide (a smooth muscle relaxant factor). The use of nitric oxide inhibitors, in our respiratory models, could determine whether glucan (BY) could act through release of nitric oxide.

References

Constantine J.W. 1965. The spirally cut tracheal strips preparation. Journal of Pharmacy and Pharmacology, 17: 384-385.

Jones A. and Nicholls P.J., 1998. Can a particulate, insoluble (1→3)-β-D-glucan cause general hypo-responsive activity in guinea pig respiratory smooth muscle. Proceedings of the 22nd Cotton and Other Organic Dusts Research Conference, Beltwide Cotton Conferences, 254-258.

Ljungman A.G., Leanderson P. and Tagesson C., 1998. (1→3)-β-D-Glucan stimulates nitric oxide generation and cytokine mRNA expression in macrophages. Environmental Toxicology and Pharmacology, 5: 273-281.

McMillan R. M. and Walker E.R.H., 1992. Designing therapeutically effective 5-lipoxygenase inhibitors. Trends in Pharmacological Sciences, 13: 323-330.

Yamane K. and Kobayashi T., 1990. Endogenous AA metabolites and their possible role in tracheal smooth muscle tone in guinea pigs. Journal of Applied Physiology, 69 (1): 26-32.

Acknowledgements

PJN is grateful to the British Cotton Growing Association Ltd: Work People's Collection Fund for financial support.

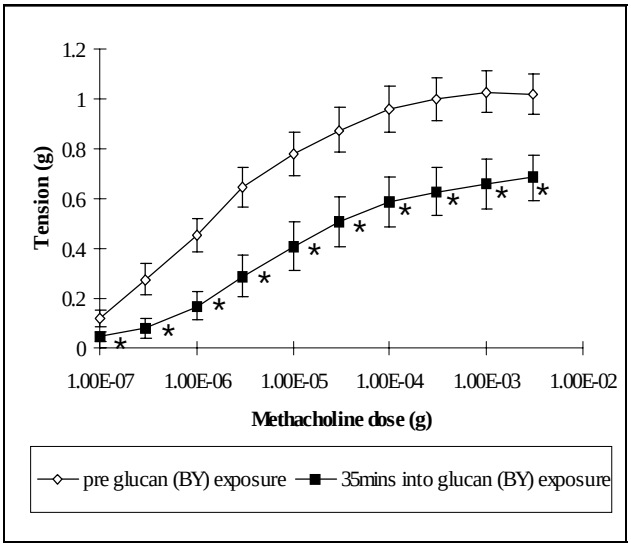

Figure 1: Typical control trachea - Change in reactivity to methacholine of a guinea pig tracheal spiral after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=6, *P < 0.05, **P = 0.05

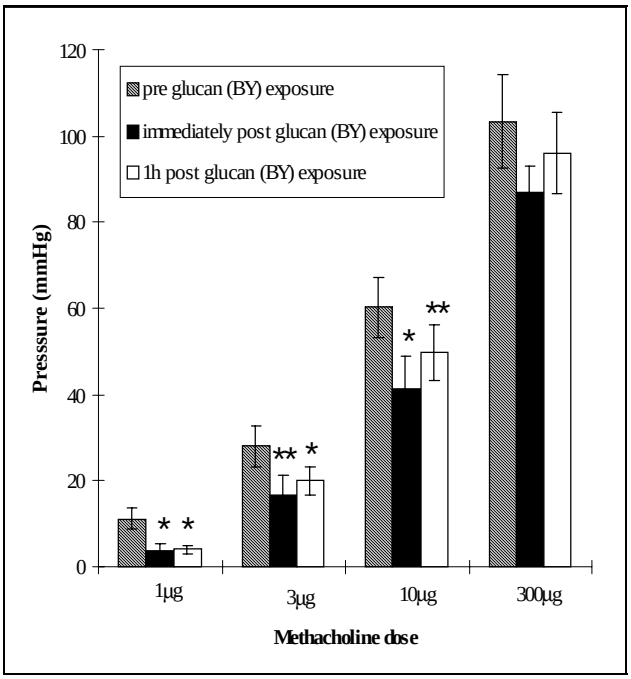

Figure 2: Typical control lung - Change in reactivity to methacholine of a guinea pig perfused lung after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=7, *P < 0.05, **P = 0.05

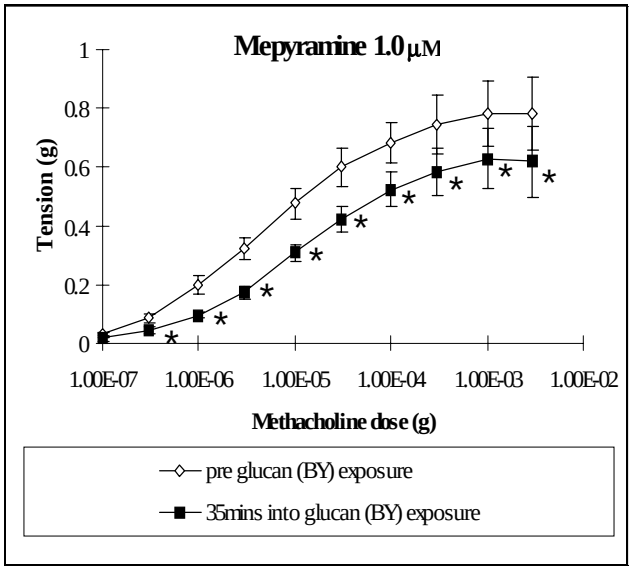

Figure 3: Change in reactivity to methacholine of a guinea pig tracheal spiral, in the presence of mepyramine 1.0µM, after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=6, *P < 0.05, **P = 0.05

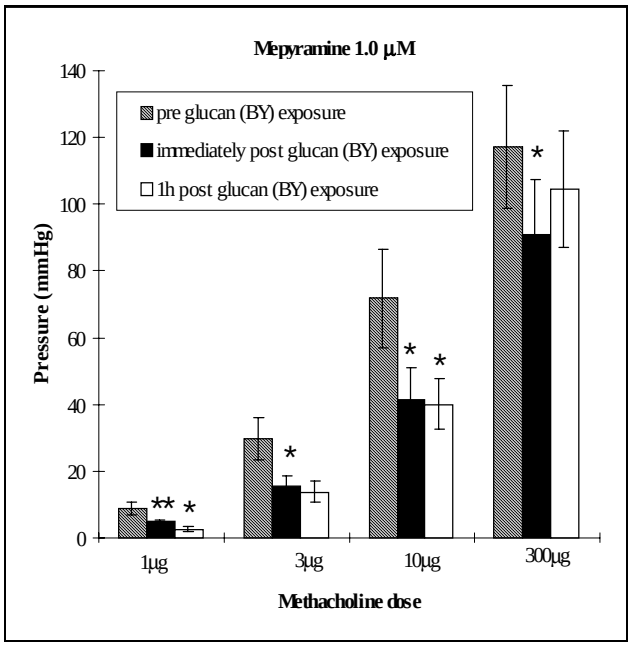

Figure 4: Change in reactivity to methacholine of a guinea pig perfused lung, in the presence of mepyramine 1.0µM after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=6, *P < 0.05, **P = 0.05

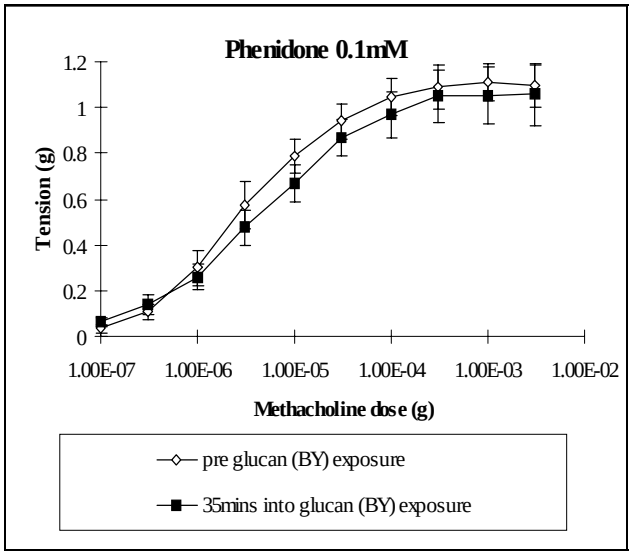

Figure 5: Change in reactivity to methacholine of a guinea pig tracheal spiral, in the presence of phenidone 0.1mM, after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=4, *P < 0.05, **P = 0.05.

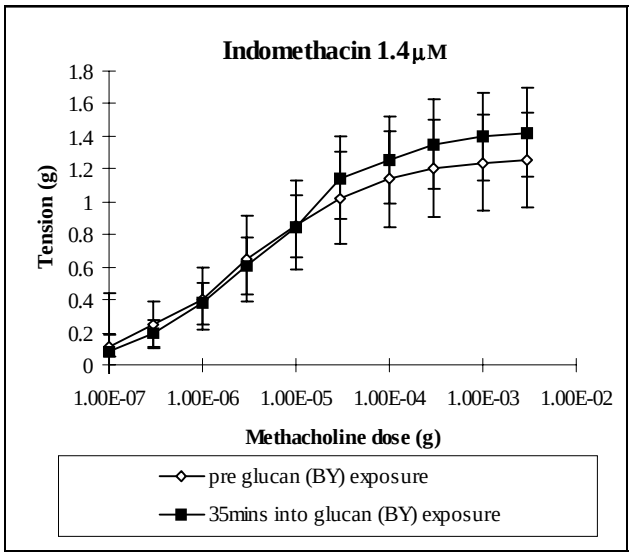

Figure 6: Change in reactivity to methacholine of a guinea pig tracheal spiral, in the presence of indomethacin 1.4µM, after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=5, *P < 0.05, **P = 0.05.

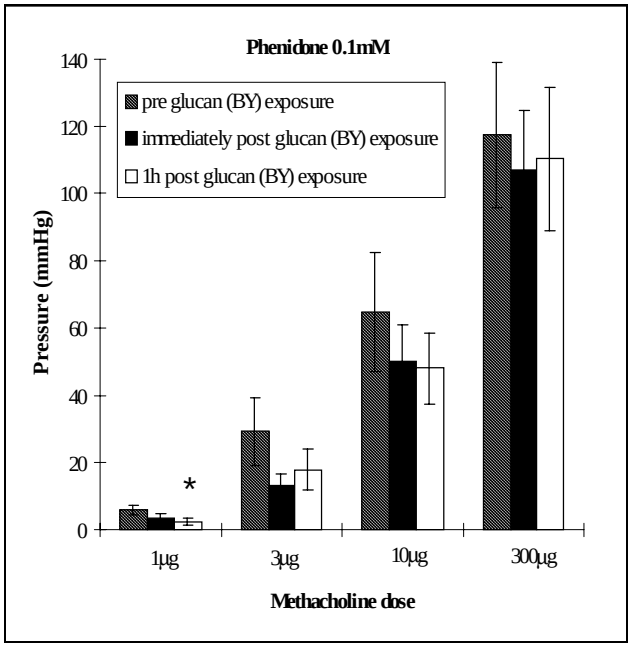

Figure 7: Change in reactivity to methacholine of a guinea pig perfused lung, in the presence of phenidone 0.1mM after exposure to 50µg ml^{-1} glucan (BY). Values are means ± s.e.m, n=7, *P < 0.05, **P = 0.05

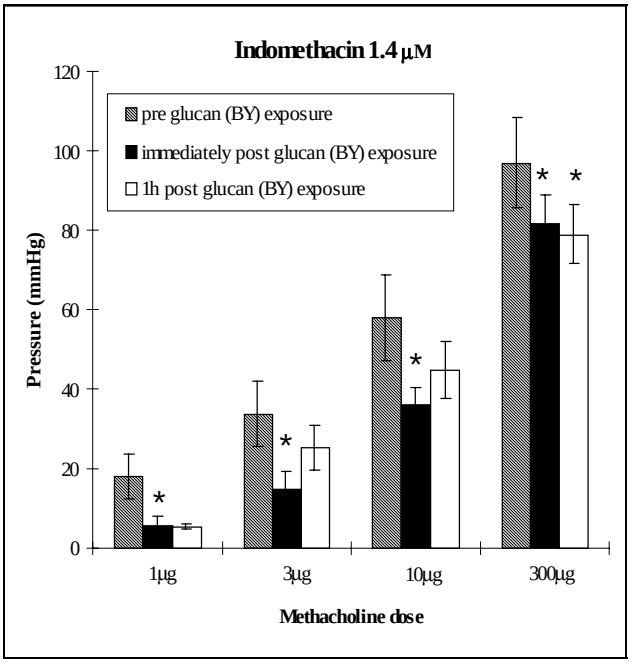

Figure 8: Change in reactivity to methacholine of a guinea pig perfused lung, in the presence of indomethacin 1.4µM after exposure to 50µg ml^{1} glucan (BY). Values are means ± s.e.m, n=7, *P < 0.05, **P = 0.05