CAN A PARTICULATE, INSOLUBLE (1→3)-β-D-GLUCAN CAUSE GENERAL HYPO-RESPONSIVE ACTIVITY IN GUINEA PIG RESPIRATORY SMOOTH MUSCLE?

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Abstract

There are two major mechanisms by which a contraction can be mediated in airway smooth muscle, namely either a receptor-operated contraction or a depolarisation-induced contraction. Glucan from baker's yeast, an insoluble, particulate glucan, can induce hypo-responsiveness to methacholine-induced (a receptor-operated contractile agent) contractions in guinea pig respiratory smooth muscle. The present experiment was designed to determine whether this effect was either specific to methacholine, or part of a more general hypo-responsive phenomenom. Using immersed trachea and pefused lung tissue models, contractions were produced with one of a number of constrictor agents. The effect of exposure of the airway smooth muscle to 50µg/ml glucan (from baker's yeast) on these responses was then determined. This glucan caused some degree of hypo-responsiveness to all contractile agents used. This suggests that baker's yeast glucan acts by a non-specific mechanism.

Introduction

Contractile mechanisms in airway smooth muscle can be categorised as either receptor-operated contractions, or depolarisation-induced contractions (Knox and Tattersfield, 1994). The majority of contractile agents, such as methacholine and histamine, act on specific G-protein linked receptors. This binding causes activation of the inositol phospholipid second messenger system and release of intracellular calcium (Hall and Chilvers, 1989) (Figure 1).

However, certain agents, such as tetraethylammonium and potassium chloride (KCl) cause membrane depolarisation. This promotes the influx of calcium into the cell via voltage dependent calcium channels (Knox and Tattersfield, 1994; Foster et al., 1983). This externally derived calcium binds to calmodulin and this complex activates myosin light chain kinase (Figure 1). The contraction is then produced by phosphorylation of myosin light chain, allowing cross bridge formation, and finally contraction.

Glucan from baker's yeast (glucan (BY)), an insoluble particulate (1→3)-β-D-glucan, has been shown to cause a decrease in reactivity to methacholine of the guinea pig perfused lung (Jones and Nicholls, 1997). It causes a significant hypo-responsive effect on this receptor-operated contractor agent, with an accompanying reduction in the maximal response of the tissue. These results imply that the efficiency of the methacholine/M3 receptor/2nd messenger system is reduced by the action of the glucan.

The aim of the present experiments was to determine whether the action of glucan (BY) was either specific to methacholine-induced bronchoconstriction or whether it also affected other constrictor agents. A range of constrictors from both mechanism categories was chosen to assess the specificity of glucan (BY), and to investigate its possible mechanism of action.

Materials

All chemicals were purchased from Sigma Chemicals.

Glucan (BY) was dissolved in distilled water to a concentration of 1mg/ml. It was then sonicated, using an MSE Soniprep 150, for 5 minutes to aid solubility. Finally, this stock solution was diluted to the required concentration of 50µg/ml with Krebs solution.

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 tissue and blood vessels. Then, at the bifurcation, the trachea was cut and the lungs were separated into right and left lobes.

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 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

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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, before the determination of a dose-response relationship to one of four constrictors (methacholine, histamine, U46619 or KCl). In the case of the trachea a full dose-response curve was performed, However, with the lungs, only four doses that spanned the range of a standard dose-response curve to the constrictor were used.

The lungs were then perfused with 50µg/ml glucan (BY) for 1 hour. After exposure to the glucan, the lungs were reperfused with normal Krebs solution. Immediately and 1 hour after reintroducing the Krebs solution, the dose-response relationship to the particular constrictor was repeated to assess any changes in reactivity.

The tracheal spirals were immersed in 50µg/ml glucan (BY). After 35 minutes, and still in the presence of the (1→3)-β-D-glucan, the dose response curve to the constrictor 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 glucan (BY) 50µg/ml significantly reduced the reactivity of the guinea pig perfused lung and immersed trachea to methacholine and histamine at all doses (Figures 2-5). The maximum response to each of the constrictors was also reduced after exposure to the (1→3)-β-D-glucan. Only in the case of methacholine was the reduction in response sustained over a 1h period.

When U46619 (a thromboxane A2 analogue) was employed as the constrictor, the perfused lung showed a hypo-responsive effect after exposure to glucan (BY) (Figure 6). Again here, the maximal response of the tissue to U46619 was also reduced However, in the trachea, although there was a trend for a decrease in reactivity to U46619 the responses were not significantly different from those pre glucan (BY) exposure (Figure 7).

After immersion in glucan (BY) 50µg/ml, the tracheal preparation showed a significant reduction in its response to potassium chloride (Figure 8). However, when 100mcg of KCl was administered, although there was a decrease in activity of the tissue, the result was not significant. Pefusion of the lung with the same concentration of glucan (BY) also produced hypo-responsiveness to KCl (Figure 9). A significant effect was only observed at 10mcg and 100mcg of potassium chloride. The reduction in response at these two doses was sustained over a 1h period.

There were no significant differences in the response of the control tissues to any of the constrictors over the time-course of the experiments.

Discussion

The results imply that exposure to glucan (BY) 50µg/ml causes a reduction in response to the receptor-operated constrictors methacholine and histamine. In each case there is not only a reduction in the reactivity of the tissue to the constrictor, but there is also a change in the maximum response of the tissue. This suggests that glucan (BY) affects the whole efficacy of the agonist/receptor/2nd messenger system. It does not just alter the affinity of the constrictor for its receptor.

Methacholine and histamine act on different receptors (methacholine on M3 receptors; histamine on H_1 receptors), which are highly specific for their substrate. This is further evidence for the proposal that glucan (BY) may influence the 2nd messenger system, as it would be unlikely that this (1→3)-β-D-glucan could have the same effect on two highly distinct receptors.

Glucan (BY) also seems to be capable of causing a hypo-responsive effect on contractions initiated by U46619. The mechanism of action of this constrictor is not fully understood. It is postulated that it acts on prejunctional cholinergic receptors, thus increasing release of cholinergic mediators and hence causing contraction (Barger and Evans, 1991). It is also known to act on thromboxane A2 receptors which could also cause contraction. As this (1→3)-β-D-glucan can decrease the magnitude of contractions developed by this agent, the results indicate that the glucan must act by a more non-specific mechanism.

Potassium chloride causes contractions by inducing depolarisation of the smooth muscle cell membrane, with subsequent entry of calcium into the cell. Glucan (BY) appears to be able to cause a hypo-responsive action on KCl mediated contractions in both the trachea and lungs. KCl acts much further down the contraction pathway at the calcium-calmodulin stage (Figure 1). Therefore, if glucan (BY) has an effect on this constrictor it must be acting at this stage or beyond. It could be affecting myosin light chain kinase, or inhibiting the phosphorylation of myosin light chain.

However, a more plausible explanation is that glucan (BY) is acting on a completely different pathway. In order to maintain an equilibrium there is a relaxatory pathway that acts in opposition to the constriction mechanisms in airway smooth muscle. It is possible that glucan (BY) is activating the relaxation pathway to produce a general hypo-responsive action on various constrictors. One report suggests that (1→3)-β-D-glucans can activate protein kinase A (Williams et al., 1996). This would be consistent with the present

work, as protein kinase A is the main enzyme involved with relaxation of airway smooth muscle.

These important findings significantly add to the understanding of the mechanism of action of glucan (BY) on respiratory smooth muscle. To further clarify the conclusions, future experiments need to be use relaxatory agonists and their antagonists to determine whether the action of glucan (BY) can be mimicked or blocked.

References

Barger L.W. and Evans J.N. 1991. Airway generation-dependent induction of muscarinic hyper-responsiveness by thromboxane mimetic *in vitro*. American Review of Respiratory Disease, 144: 64-69.

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

Foster R.W., Small R.C. and Weston A.H. 1983. The spasmogenic action of potassium chloride in guinea-pig trachealis. British Journal of Pharmacology, 80: 553-559.

Hall I.P. and Chilvers E.R. 1989. Inositol phosphates and airway smooth muscle. Pulmonary Pharmacology, 2: 113-120

Jones A. and Nicholls P.J. 1997. An investigation into the effects of (1→3)-β-D-glucan on an *in vitro* guinea pig perfused lung model. Journal of Pharmacy and Pharmacology, 40 (supp. 4): 3

Knox A.J. and Tattersfield A.E. 1994. In Szekeres L, Papp J (Eds.) Pharmacology of Smooth Muscle. Springer-Verlag, Berlin. Chpt 11 - Airway Smooth Muscle: 405-444.

Williams D.L., Mueller A. and Browder W. 1996. Glucan-Based Macrophage Stimulators - A Review of their Anti-Infective Potential. Clinical Immunotherapy, 5 (5): 392-399.

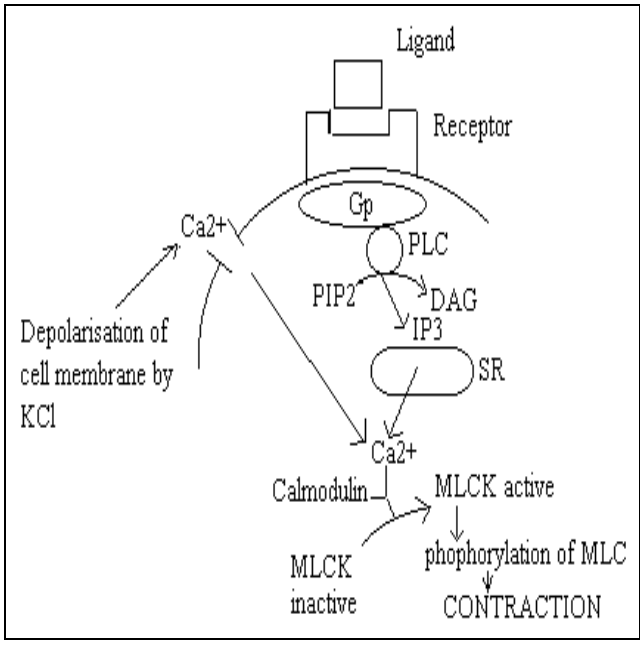

Figure 1: Contraction mechanisms in airway smooth muscle. Gp = G protein, PLC = phospholipase C, PIP2 = inositol biphosphate, IP3 = inositol 1,4,5, triphosphate, DAG = diacylglycerol, SR = sacroplasmic reticulum, MLCK = myosin light chain kinase, MLC = myosin light chain.

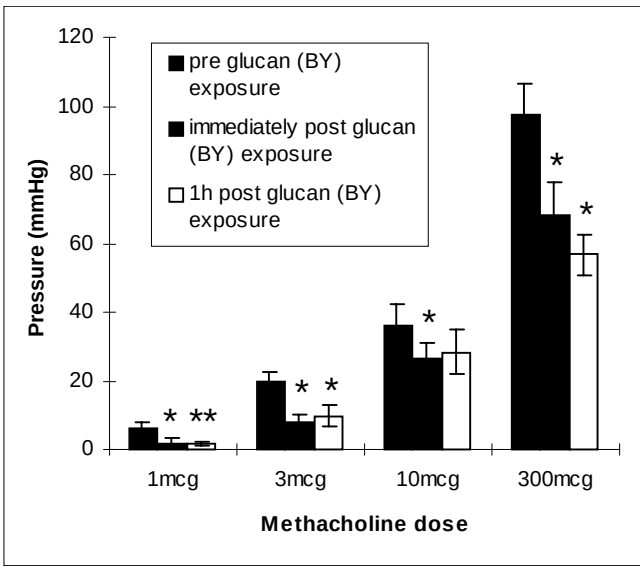

Figure 2: Change in reactivity to methacholine of a guinea pig perfused lung preparation after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * $P < 0.05$, ** $P = 0.05$

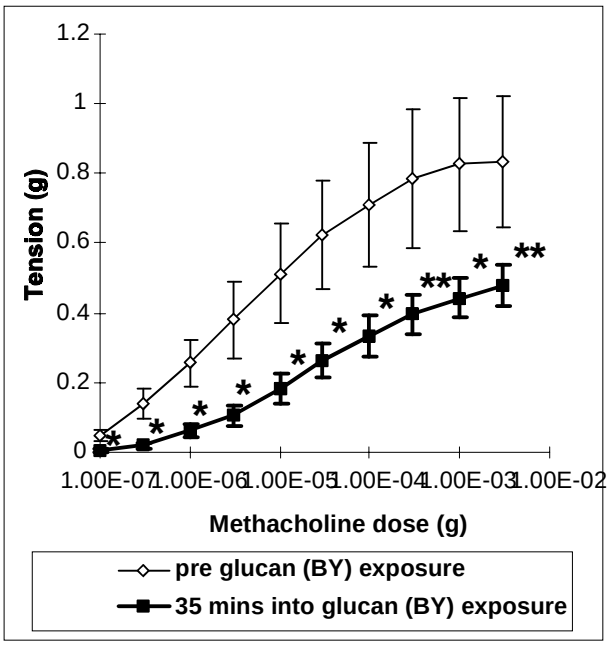

Figure 3: Change in reactivity to methacholine of guinea pig tracheal spirals after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

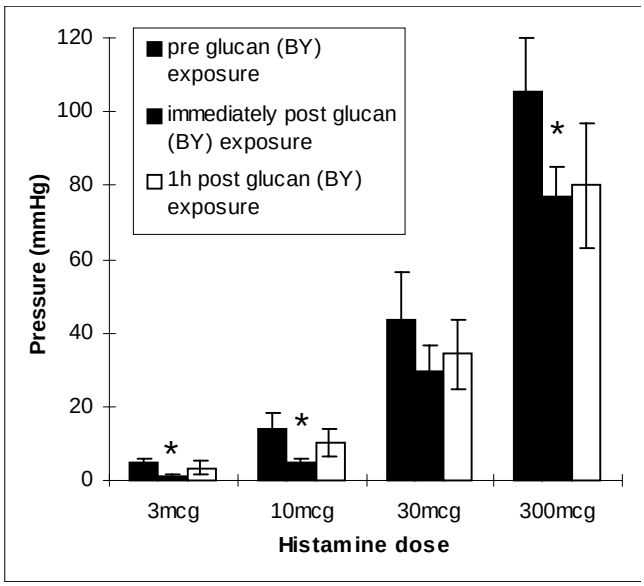

Figure 4: Change in reactivity to histamine of a guinea pig perfused lung preparation after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

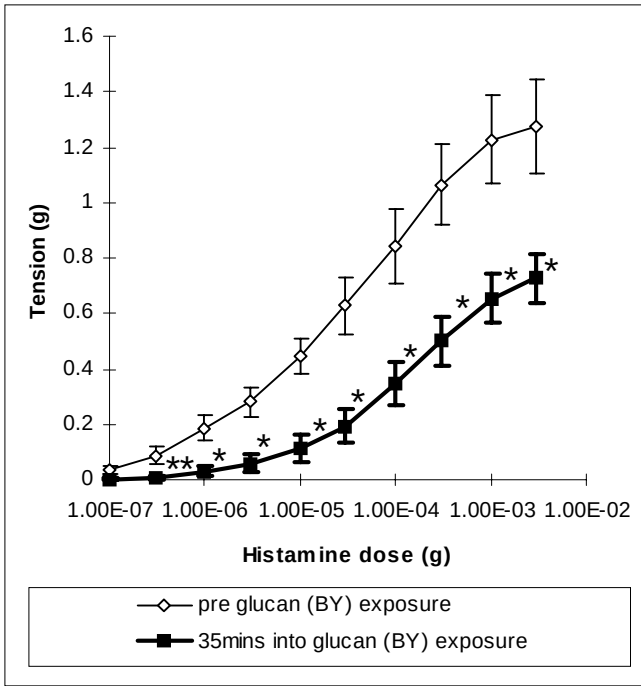

Figure 5: Change in reactivity to histamine of guinea pig tracheal spirals after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

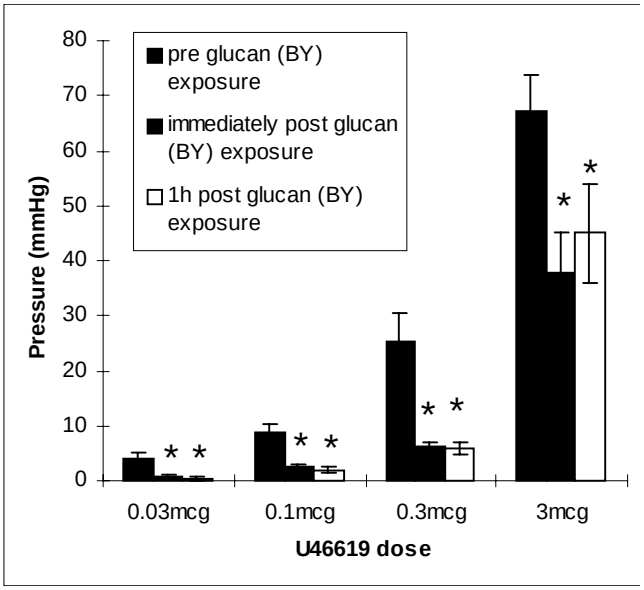

Figure 6: Change in reactivity to U46619 of a guinea pig perfused lung preparation after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

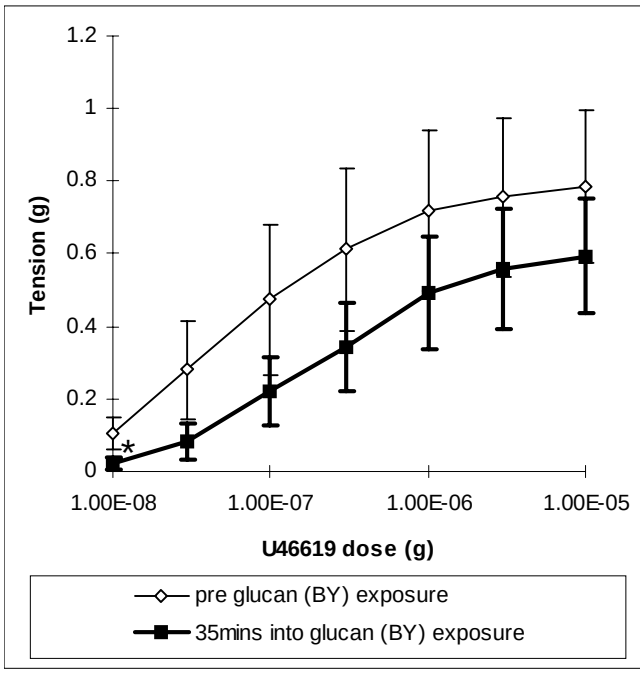

Figure 7: Change in reactivity to U46619 of guinea pig tracheal spirals after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

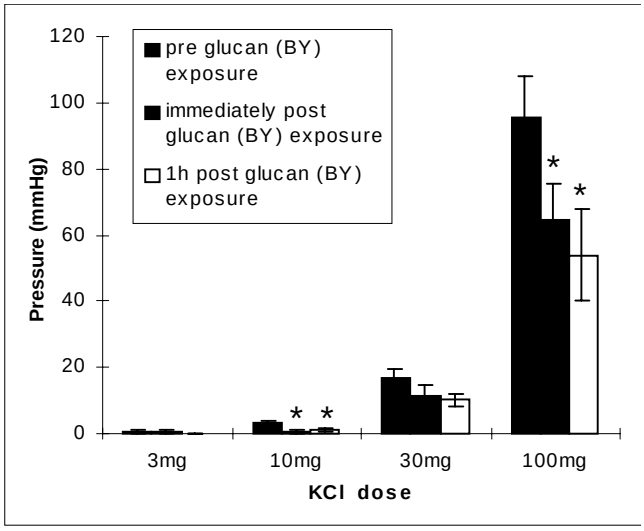

Figure 8: Change in reactivity to KCl of a guinea pig perfused lung preparation after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05

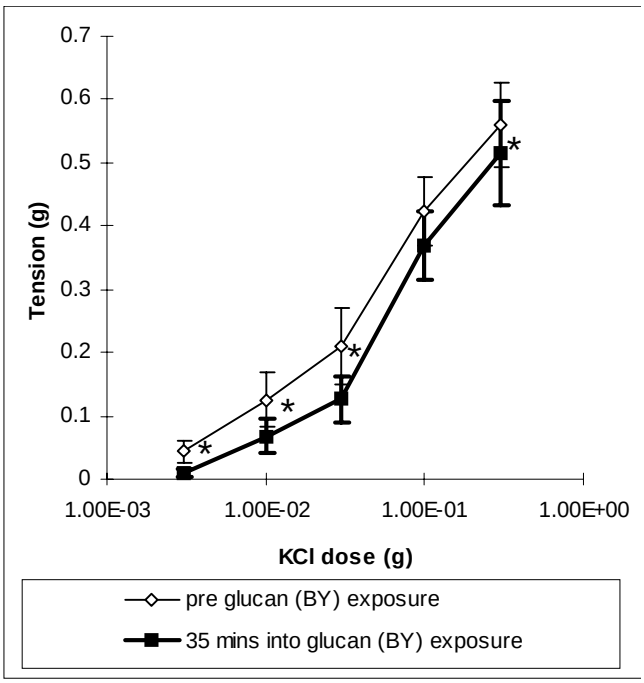

Figure 9: Change in reactivity to KCl of guinea pig tracheal spirals after exposure to glucan (BY) 50µg/ml. Values are means ± s.e.m, n=6, * P < 0.05, ** P = 0.05