THE EFFECT OF GLUCAN ON *IN VITRO* BRONCHOCONSTRICTOR RESPONSE USING GUINEA PIG TRACHEA AND PERFUSED LUNG

A. Jones, P.J. Nicholls and R.S. Young. Division of Pharmacology,
Welsh School of Pharmacy, UWCC,
Cardiff, UK.

Abstract

The (1→3)-β-D-glucans have recently come into focus as one of the possible mediators of airway changes that are observed on inhalation of organic dusts. In order to assess any changes in airway reactivity, *in vitro* experiments were set up to investigate the effect of exposure to (1→3)-β-D-glucan on bronchoconstrictor response in the guinea pig.

The work was carried out on trachea spirals and perfused lung halves. After exposure to (1→3)-β-D-glucan an increased response to the bronchoconstrictor agent was seen with the trachea spirals however, after an hour the response had returned to the pre (1→3)-β-D-glucan exposure level. The perfused lung halves showed a decrease in response after exposure to (1→3)-β-D-glucan and this was sustained over one hour.

The (1→3)-β-D-glucans do have an effect on the response of the airway to a bronchoconstrictor agent. However, at the present time it is unclear by what mechanisms it might act.

Introduction

Glucans are polymers of glucose found in the cell walls of plants, moulds, fungi and bacteria. Of particular interest are the (1→3)-β-D-glucans present in several organic dusts, such as cotton and grain. These glucans are derived predominantly from fungi and have potent immunobiological effects (1).

The (1→3)-β-D-glucans consist of glucopyransoyl subunits connected by (1→3)-β polyglucoside linkages to form polymer chains. The chains can exist in a variety of configurations from random coils to branched triple helices (1).

The (1→3)-β-D-glucans have been associated with pulmonary 'sick building ' symptoms, e.g. dry cough and nasal irritation (1) and elevated levels of glucan have been reported in 'sick buildings' (2). Rylander has reported a potential relationship between the concentration of glucan found in sick buildings and the severity of respiratory symptoms that occur (3).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:166-169 (1997)
National Cotton Council, Memphis TN

In order to investigate these results, experiments have taken place to assess the toxicity of (1→3)-β-D-glucans on the airways. Particular attention has focused on its role in inflammation (1,4,5,6,7). Presently, it appears that on exposure of guinea pigs to (1→3)-β-D-glucan, only the water soluble glucans cause any inflammatory activity (1,4,5,6,7). However, the water insoluble group do provoke an inflammatory response (1→3)-β-D-glucan when they are in combination with endotoxin (1,4,5,6,7).

The following experiment was designed to look at another possible aspect of (1→3)-β-D-glucan activity - airway reactivity. It investigates the effects of this agent on the ability of isolated tissues to respond to a bronchoconstrictor agent.

Method

Materials

Methacholine chloride and glucan (from Baker's Yeast) were obtained from Sigma Chemicals UK Ltd.

The glucan was mixed in deionised, distilled water to achieve a concentration of 1mg ml^{-1}. This was then sonicated to produce a slightly soluble mixture.

Animals

Male Dunkin Hartley guinea pigs, weight range 300 to 500g were used throughout the experiments. They were housed in approved holding units where tap water, oranges and pelleted food were freely available. The temperature and humidity of the housing area was maintained at 21◦C, 50% respectively.

Experimental procedure

The animals were sacrificed by cervical dislocation and exsanguination. The trachea, heart and lungs were removed into 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) After removal of the heart, the lungs and trachea were cleaned of extraneous tissue and blood vessels. Then at the bifurcation, the trachea was cut and the lungs were separated into right and left lobes.

The same experimental set up was used as described by Young and Nicholls(8) Each lung was attached, via the bronchi, to a cannula using surgical cotton. The lungs were then perfused, using a peristaltic pump, with Krebs at a rate of 5ml/min. Pressure changes 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 (9). Each spiral was then suspended in an organ bath filled with Krebs. The contractions in the trachea were measured by a Dynamometer UFI isometric transducer attached to the same Devices MX4 recorder.

In both the lungs and the trachea, the temperature of the Krebs was maintained at 35◦C with a circulator, and oxygenated by passing gas (95% O_2, 5% CO_2) though it.

One lung and one trachea half were used as time controls, to monitor reactivity changes in normal tissue.

The tissues were allowed to equilibrate for an 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 typical dose response curve to methacholine were used. These were chosen on the basis of previous work that had been done in this laboratory.

The glucan mixture (1mg ml^{-1}) was then added to Krebs to give an almost clear solution of 50μg ml^{-1}. This solution was then added to the organ bath of the trachea and perfused through the lung for 1 hour. Immediately and 1 hour after replacing these solutions with pure Krebs, the dose response relationships were repeated to assess any changes in reactivity.

In order to eliminate any apparatus bias, 'cross over' experiments were undertaken, i.e. the 'test' tissue was in organ bath or cannula A for the first experiment and organ bath or cannula B for the second.

Results

Perfused lung halves

The dose response relationship for the lung preparation was altered after perfusion with (1→3)-β-D-glucan at a concentration of 50μg ml^{-1}. At 3μg of methacholine a significant decrease in response was observed post exposure to (1→3)-β-D-glucan (Figure 1). One hour after exposure to (1→3)-β-D-glucan the decrease in response to methacholine was still significant. The same relationship was seen at a dose of 300μg, suggesting that the maximum response of the tissue was also reduced. Figure 2 (the control preparation) indicates that time did not effect the reactivity of the tissue, as no significant differences were detected.

Trachea spirals

The tracheal tissue showed a different relationship to the perfused lungs. Again, there were no changes of reactivity with time in normal tissue, the control showing no significant differences between the repeated dose response curves (Figure 3).

However, after exposure to (1→3)-β-D-glucan, there was a change in the response of the trachea. From 10μg ml^{-1} to the maximum response at 3mg ml^{-1} of methacholine, the reactivity of the tissue, immediately post exposure of (1→3)-β-D-glucan, was significantly greater (Figure 4). The large error bars at these doses indicates the range of responses due to biological variation within the group. Nevertheless, despite these variances, each time a dose of methacholine ($\geq$10μg ml^{-1}) was administered post-exposure there was always an increase in response from the pre-exposure level. This is clarified in Figure 5. Here the difference between reactivity post-exposure and pre-exposure was plotted; it shows that although there was a large variation of responses within a group, the change in response that was repeatedly observed post-exposure fell within narrower limits.

When reactivity was looked at 1 hour post (1→3)-β-D-glucan exposure there were no significant differences from the pre-exposure dose response curve (Figure 6). The increased response that was seen immediately post-exposure was lost within the hour.

Discussion

The results from this pilot study suggest that (1→3)-β-D-glucan does, at least, have an effect in the airways *in vitro*. It appears that this agent different resultant effects in the trachea, where it causes increased reactivity, compared to the lung, where there is a decrease in response. The mechanism of action of (1→3)-β-D-glucan is still unclear, but this study implies that the action maybe non-specific.

In both the tracheal and lung preparations, there was an alteration of the maximal response, indicating that the (1→3)-β-D-glucan might change the intrinsic efficacy of the methacholine receptor system. In the case of the trachea it increases it, while in the lung there is a reduction in efficacy.

Endotoxin is one of the most widely studied agents in organic dusts. A previous *in vitro* study, using the same model, showed that for the dose response curve to methacholine, of both the lung and trachea, shifted significantly to the left after treatment with endotoxin (8). However, there was no signifcant change of the maximal response. This suggests that endotoxin and (1→3)-β-D-glucan work by different machanisms of action.

Organic dusts are composed of many different constituents Therefore, it is probable that it is the interaction of these that elicits the airway responses that are observed on inhalation of these agents. This study indicates that the (1→3)-β-D-glucan has the potential to act both synergistically and antagonistically with dust components, such as endotoxin.

The results imply that it would be worthwhile further investigating the *in vitro* effects of (1→3)-β-D-glucan. It would be beneficial to build up a profile, with respect to the significance of the dose, solubility and time of exposure of (1→3)-β-D-glucan. Also, it would be useful to determine the reactivity of trachea and lung isolated from guinea pigs exposed to (1→3)-β-D-glucan by inhalation.

References

1. Williams DL, 1996. Overview of (1→3)-β-D-glucan chemistry, immunology and toxicology. *Cotton and Other Organic Dusts: Proceedings of the 20th Cotton and Other Organic Dusts Research Conference*: 285-288. Beltwide Cotton Conferences.

2. Goto H, Yuasa K, Rylander R, 1994. (1→3)-β-D-glucan in indoor air, its measurement and in vitro activity. *American Journal of Industrial Medicine*; **25**: 81-83.

3. Rylander R, Persson K, 1991. Sick Building symptoms and levels of airborne glucan. *Cotton Dust: Proceedings of the 15th Cotton Dust Research Conference*: 236-237. Beltwide Cotton Conferences.

4. Fogelmark B, Sjostrand M, Rylander R, 1994. Pulmonary inflammation by repeated inhalations of (1→3)-β-D-glucan and endotoxin. *Internatinal journal of experimaental Pathology*; **75** (2): 85-90.

5. Rylander R, Fogelmark B, 1994. Inflammatory Responses by Inhalation of Endotoxin and (1→3)-β-D-glucan. *American Journal of Industrial Medicine*; **25** (1): 101-102.

6. Rylander R, 1994. Pulmnary Immuntoxicology of inhlaed (1→3)-β-D-glucan. *Cotton Dust: Proceedings of the 18th Cotton Dust Research Conference*: 347-349. Beltwide Cotton Conferences.

7. Fogelmark B, Goto H, Yuasa K, Marchat B, Rylander R, 1992. Acute Pulmaonary toxicity of inhaled β-(1,3)-glucan and endotoxin. *Agents Actions*; **35** : 51-56.

8. Young R.S, Nicholls PJ 1996. Airway responses to Bronchoconstrictor Agents in the Guinea-pig Perfused Lung after Exposure to Escherchia Coli Lipopolysaccharide - An In Vitro Model for Hyper-responsiveness. *Pharmaceutical Sciences*, **2**: 77-81.

9. Constantine JW, 1965. The spirally cut tracheal strips preparation. *Journal of Pharmaceutical Pharmacology*, **17**: 384-385.

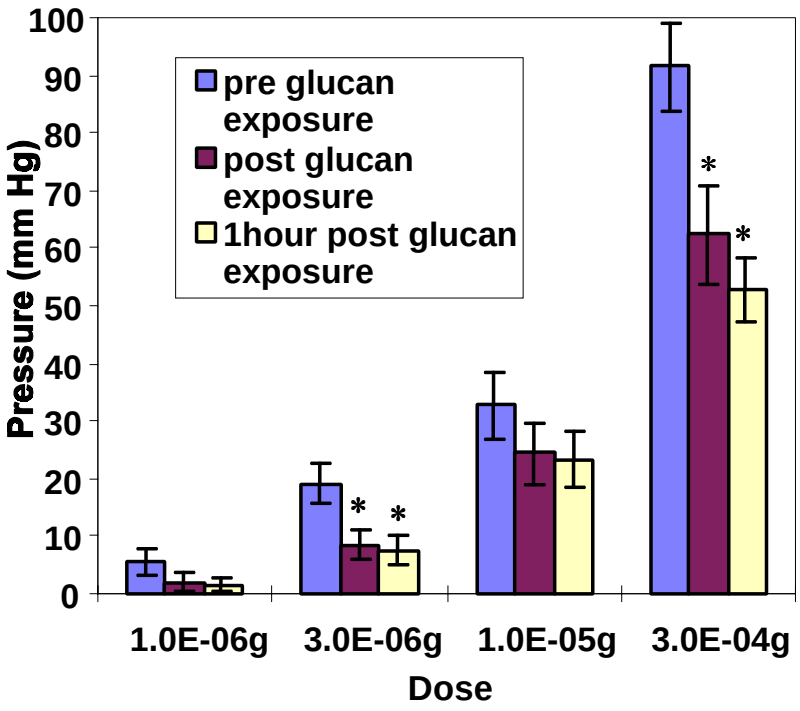

FIGURE 1: The change in reactivity to methacholine, of perfused lung half, after exposure to (1→3)-β-D-glucan. Values are means ± s.e.m., n=5; paired Student's *t*-test; $*P < 0.05$.

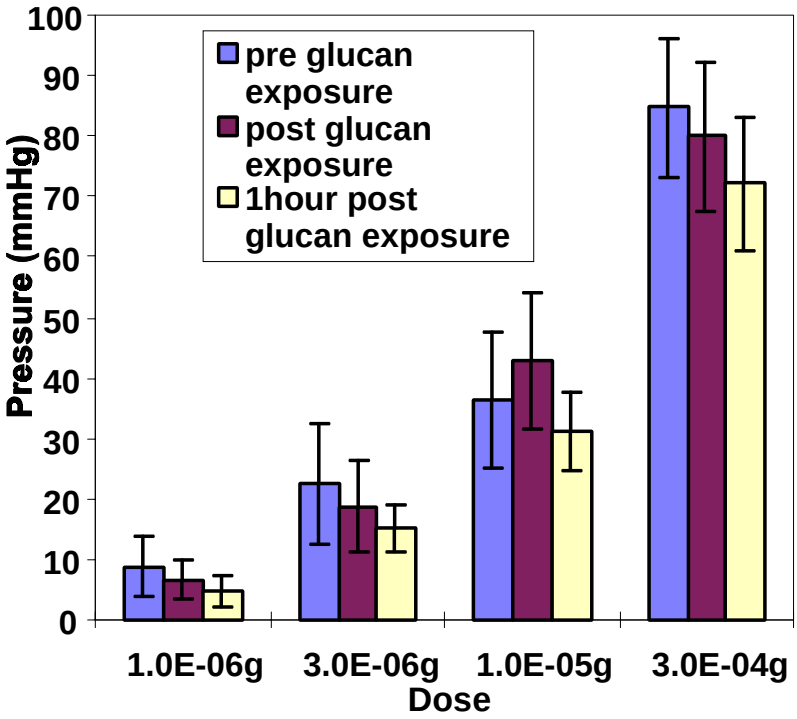

FIGURE 2: The change in reactivity to methacholine, of control perfused lung, as a function of time. Values are means ± s.e.m., n=5.

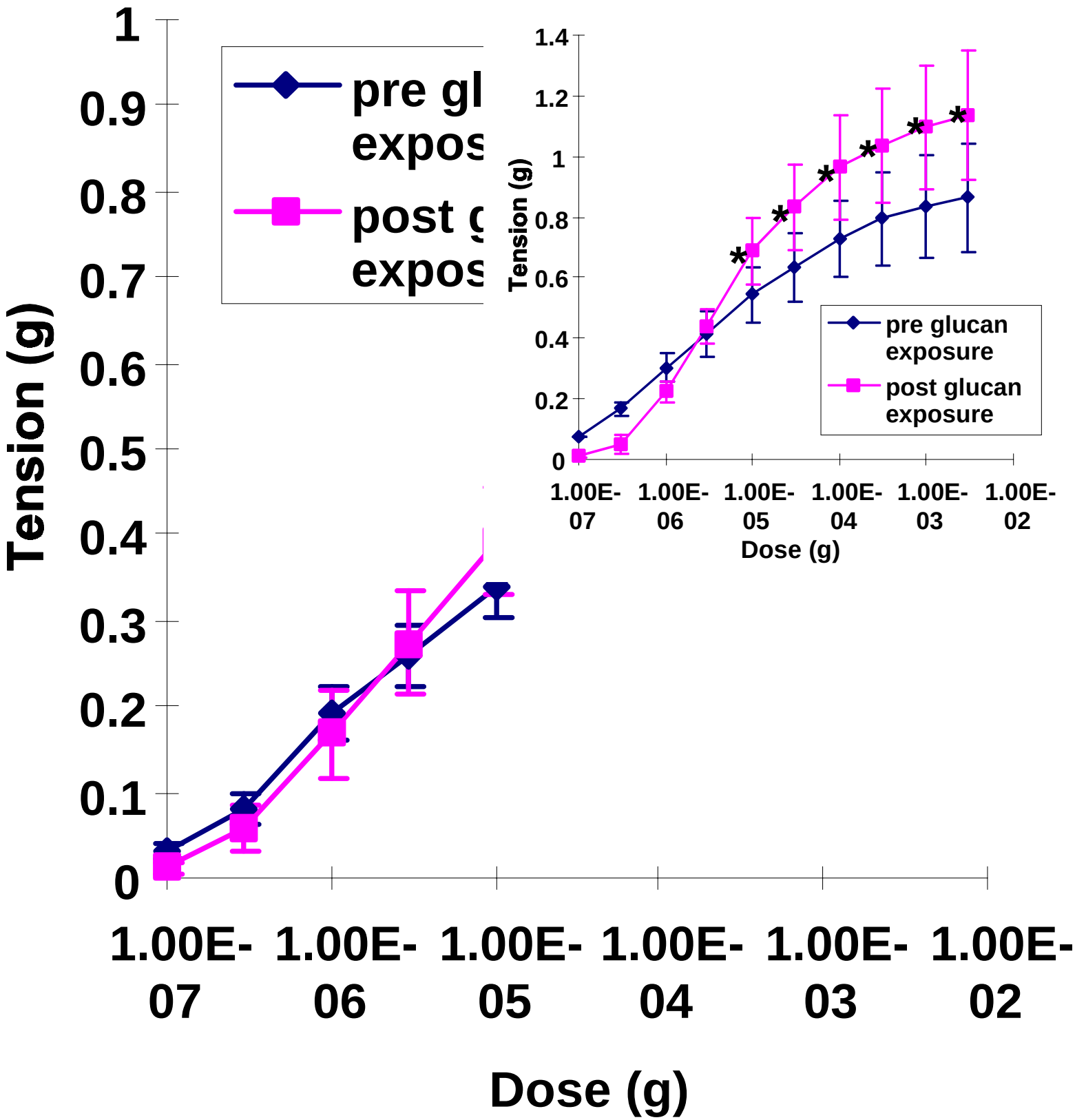

FIGURE 3: Change in reactivity to methacholine, of control trachea spirals, as a function of time. Values are means ± s.e.m, n=5.

FIGURE 4: Changes in reactivity of trachea spirals to methacholine, immediately post (1→3)-β-D-glucan exposure. Values are means ± s.e.m., n=5. Pairs students *t*-test. * $P < 0.05$

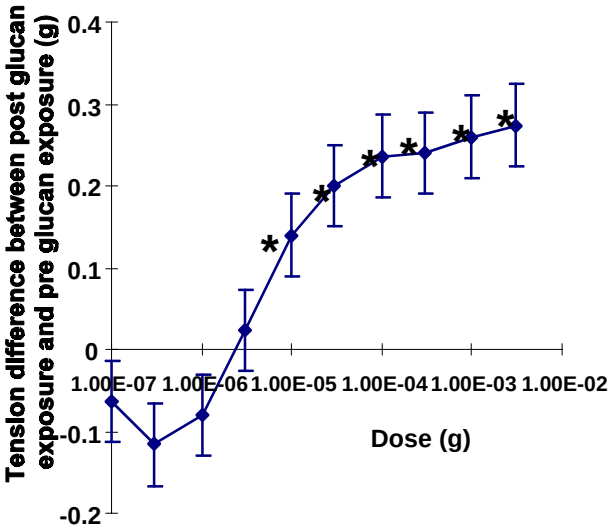

FIGURE 5: The difference in reactivity (to methacholine), of trachea spirals, between post (1→3)-β-D-glucan exposure and pre (1→3)-β-D-glucan exposure. Values are means ± s.e.m., n=5;
* significant results from Figure 4.

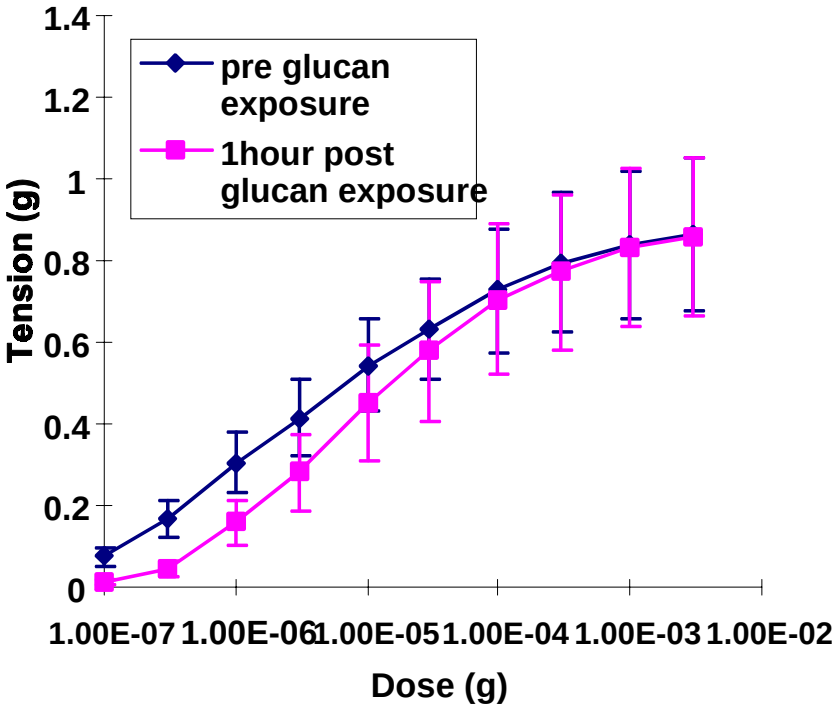

FIGURE 6: Changes in reactivity of trachea spirals to methacholine, 1 hour after (1→3)-β-D-glucan exposure. Values are means ± s.e.m, n=5.