In vitro effect of toluene diisocyanate on beta adrenergic and muscarinic receptor function in lung tissue of the rat

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ABSTRACT To investigate the role of pharmacological mechanisms in toluene diisocyanate (TDI) induced occupational asthma, the effects of TDI on rat trachea ring and lung parenchymal strip were studied in vitro. The most prominent effect observed was a stimulation of metacholine ($1\mu\text{M}$) induced contraction of the tracheal ring by $1\mu\text{M}$ TDI (added in dimethyl sulphoxide). The results were less pronounced when TDI was added from a stock solution prepared in water, which is possibly due to (co)polymerisation. It is concluded that the pharmacological effect of TDI may result from an autonomic imbalance between cholinergic and B-adrenergic neural control.

Toluene diisocyanate (TDI), a highly reactive chemical used as a polymerising agent in the production of polyurethane foams, plastics, and adhesives, is known to cause bronchial asthma in some of those exposed to it.¹⁻³

Immediate, late, and dual type asthma may develop even in response to low concentrations of TDI (less than 0.001 ppm).⁴ Asthma is believed to develop in about 5% of workers regularly exposed to TDI, and most have been non-atopic.^{1,5} The underlying cause of isocyanate asthma is not understood, although immunological and pharmacological mechanisms have been proposed.⁶⁻¹⁰

Without questioning the immunological mechanisms involved we were puzzled with the contradictory pharmacological in vivo and in vitro data. The early observation that almost half of TDI bronchoprovocation positive patients failed to show manifestations of histamine hyperreactivity⁴ led Bernstein to the suggestion that "under appropriate conditions that cannot be defined precisely at the present time, diisocyanate compounds may act as direct pharmacological agonists or as inducers of non-specific bronchial hyperreactivity."¹⁰

The idea that neural control may be abnormal in asthma¹¹ generated the design of the present investigation: elucidating the effect of TDI on muscarinic and beta-adrenergic receptor function using two different experimental systems: isolated rat trachea ring and the

lung parenchymal strip. We used the laryngeal portion of the trachea which is well innervated and the lung parenchymal strip which is poorly innervated.

Material and methods

CHEMICALS

TDI was purchased from Merck (art No 808264) and was a mixture of 2, 4-TDI (80%) and 2,6-TDI (20%). (-) isoprenaline hydrochloride and metacholine chloride were products of Sigma Chemical Co, St Louis, Mo, USA. All other reagents used were reagent grade.

METHODS

Male Wistar rats (240-330 g, TNO Zeist, The Netherlands) were killed by a blow on the head and bled. The organs were rapidly excised and after preparation mounted in a water jacketed organ bath at a temperature of 37°C . The bath contained Krebs buffer ($\text{NaCl } 117.5\text{ mM}$, $\text{KCl } 5.6\text{ mM}$, $\text{MgSO}_4\text{ } 1.18\text{ mM}$, $\text{CaCl}_2\text{ } 2.5\text{ mM}$, $\text{NaH}_2\text{PO}_4\text{ } 1.28\text{ mM}$, $\text{NaHCO}_3\text{ } 25.0\text{ mM}$, and glucose 5.5 mM) which was gassed with a mixture of 95% O_2 and 5% CO_2 to maintain oxygen tension and a pH of 7.4.

The TDI used in all experiments was prepared fresh before the start of each experiment and dilutions were made in water or in dimethyl sulphoxide (DMSO). In the latter case control experiments were performed with an equal amount of DMSO that did not exceed a final concentration of 1% DMSO (v/v) in the organ bath.

Fig 1 Concentration dependent relaxation by (-)isoprenaline on trachea preparation of rat after contraction by $10^{-6} M$ (1), $3 \cdot 10^{-6} M$ (2), and $10^{-5} M$ (3) metacholine. Precontraction by metacholine has been depicted as 100%. **Insert:** Cumulative dose response curve of metacholine. All curves are mean of at least three separate experiments.

Fig 2a and b Effect of TDI (dissolved in DMSO) on precontraction induced by metacholine ($10^{-6} M$) and subsequent isoprenaline concentration dependent relaxation at TDI concentrations of 0 (1), $10^{-6} M$ (2), $10^{-5} M$ (3), and $10^{-4} M$ (4). **Figure 2b** as in 2a, except that TDI was dissolved in water just before administration to organ bath. All curves are mean of at least two separate experiments.

Single trachea rings cut at the site opposite to the muscles were used. A passive force of 0.4 g was applied and recording was performed isotonicly using a Hugo Sachs TL-2 Hebelaufnehmer. After an equilibrium period of 40 minutes with four intermediary washings either a cumulative dose response curve of metacholine or a cumulative dose response curve (-)isoprenaline (after precontraction for 15 minutes with metacholine at a concentration indicated) was recorded. At the start of each experiment two dose response curves with (-)isoprenaline were obtained, the second serving as a control. Between each curve two rapid washings with a subsequent washing period of 30 minutes with three intermediate washings was applied. After the second curve the trachea preparation was incubated with TDI for 30 minutes; subsequently the metacholine precontraction was induced (15 min) and a cumulative dose response curve for isoprenaline was recorded.

Parenchymal strips (isotonically recorded, with a passive force of 0.4 g) were equilibrated for 40 minutes in Krebs buffer as described above, during which four intermediate washings were performed. Cumulative dose response curves were obtained as described for the tracheal ring according to Kramer *et al.*¹²

Fig 3 Absence of any effect of TDI (10^{-4} M dissolved in DMSO) on precontraction of trachea ring induced by 10^{-6} M metacholine and subsequent isoprenaline line concentration dependent relaxation. ● indicates control experiment (DMSO only) and □ indicates relaxation in presence of 10^{-4} M TDI. Curves are mean of at least two separate experiments.

Results

TRACHEAL SMOOTH MUSCLE

To obtain a maximal relaxation effect in isolated trachea ring, we established the effect of precontraction on maximal relaxation. Precontraction was tuned by varying metacholine concentrations (fig 1). The insert in fig 1 shows the concentration response relation for metacholine. From fig 1 it is clear that when increasing the precontraction of the trachea ring, the pD_2 value (logarithm of the isoprenaline concentration that produces a half maximal relaxation) decreases, whereas the maximal relaxation also decreases.

Figure 2 shows the effect of preincubation and presence of various concentrations of TDI added in DMSO (fig 2a) or in water (fig 2b) on the relaxation curves induced by isoprenaline in the tracheal preparation. Firstly, we observed a dose dependent activation of the muscarinic response to $1 \mu\text{M}$ metacholine. Moreover, TDI has an effect on the maximal relaxation induced by the addition of isoprenaline (fig 2a). A gradual decrease of the maximal relaxation attainable with isoprenaline is observed when the TDI concentration is increased. The maximal relaxation at 10^{-4} M TDI is about 62% of the control value.

The same qualitative effects were observed when adding TDI in aqueous solution without DMSO,

though the magnitude of the effects was considerably smaller (fig 2b).

LUNG PARENCHYMA STRIP

Experiments using the isolated lung strip failed to show any effect of TDI (10^{-6} M, 10^{-5} M, 10^{-4} M) on the half maximal contracted strip (contraction induced by $1 \mu\text{M}$ metacholine), nor was any effect detected of TDI on the isoprenaline induced relaxation of the strip brought into contraction with $1 \mu\text{M}$ metacholine (fig 3). In both experiments final concentrations of TDI in DMSO/water up to 0.1 mM were tested. Because no effects were observed only the latter concentration is shown in fig 3.

Discussion

Beta adrenergic blockade has been proposed as one mechanism by which TDI induced asthmatic reactions may be explained.^{7,13} A major reason to suppose that this is the case was the finding that TDI suppressed the stimulation of lymphocyte cAMP levels by the β -agonist isoprenaline. Moreover, McKay and Brooks, using frog erythrocytes, showed that direct stimulation of adenylate cyclase by fluoride (10 mM) was significantly reduced by TDI (1 mM).⁹ These findings suggest that impairment of the β -adrenergic response may play an important part in TDI induced bronchoconstrictive effects. Several studies have shown that all individuals who react to TDI are highly metacholine sensitive.^{8,14}

The results of this study clearly show that TDI enhances the muscarinic response to metacholine in the trachea of the rat in vitro (fig 2). The same concentrations of TDI, however, fail to produce an effect on the metacholine response in the lung parenchymal strip (fig 3). The difference between the effect of TDI dissolved in DMSO or water might be explained by (co)polymerisation of TDI in water (figs 2a, 2b) thereby leading to a decrease in the reactive concentration in the tissue. As a result, TDI dissolved in water causes effects similar to those provoked by a TDI/DMSO solution, but the extent of the response is less pronounced.

The activation of the response to metacholine in our study could be explained by the inhibitory action of TDI on acetylcholinesterase as reported.^{15,16} In addition, owing to the chemically reactive properties of TDI, a wide variety of other functionally important components mediating methacholine response may be affected. Irrespective of the ultimate cause of the increased metacholine responsiveness, it implies that a relaxation by any β -adrenergic agonist demands a higher equiactive concentration of the agonist. In fig 2 it is shown that isoprenaline relaxes the rat tracheal strip preparation previously precontracted by metacholine.

choline and that the potency of isoprenaline as a spasmolytic is inversely proportional to the strength of the contraction (achieved by varying concentrations of metacholine). Such a phenomenon (functional antagonism) results from the assumption that a higher receptor occupancy with isoprenaline is needed at higher metacholine concentrations to obtain the same effect (for detailed theoretical discussion see Kenakin¹⁷).

In addition to a change in equiactive concentration of isoprenaline at different muscle tone, the maximal attainable relaxation is also altered by the tone applied. At an increasing muscle tone the magnitude of maximal relaxation decreases (fig 1 and Kenakin¹⁷).

TDI had no effect on metacholine or isoprenaline induced responses in lung parenchymal tissue. The conditions used to tune the tracheal rings precontraction (0.4 g passive force and precontraction with 1 μ M metacholine) cause a 20% maximal contraction in this preparation. Identical experimental conditions resulted in a 50% of maximal contraction (data not shown) in the parenchymal strip.

These findings support the view that the tone applied to "contract" the preparation determines the quantitative effect of the modulator. Moreover, one should also be aware that a passive force of 0.4 g does not imply the same intrinsic tone in both preparations.

The decrease caused by TDI in relaxation induced by isoprenaline might be explained by a direct effect of TDI on the β -receptor response; in addition, the increase of the contraction caused by TDI immediately implies an apparently decreased isoprenaline potency.

In conclusion, the pharmacological effect of TDI in lung tissue may result from an autonomic imbalance. This mainly holds for the innervated portion of the lungs, the large airways. We hypothesise that neural control becomes abnormal when TDI is administered because the balance is tipped in favour of bronchoconstrictor mechanisms (increase in metacholine response) and away from bronchodilator mechanisms (diminished β -adrenergic effects—direct and indirect).

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