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

PJ A BORM,1 A BAST,2 O P ZUIDERVELD2

From the Department of Occupational Medicine,1 State University of Maastricht, 6200 MD Maastricht, and Department of Pharacochemistry,2 Faculty of Chemistry, Free University, 1081 HV Amsterdam, The Netherlands

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 μM) induced contraction of the tracheal ring by 1 μM 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.1,2,3 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.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."11

The idea that neural control may be abnormal in asthma12 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 of reagent grade.

METHODS Male Wistar rats (240–330 g, TNO Zeist, The Netherlands) were killed by a blow on the head and bleeding. 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 (NaCl 117.5 mM, KCl 5.6 mM, MgSO4 1.18 mM, CaCl2 2.5 mM, NaH2PO4 1.28 mM, NaHCO3 25.0 mM, and glucose 5.5 mM) which was gassed with a mixture of 95% O2 and 5% CO2 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.

Accepte 4 January 1988
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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 isotonically 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 Log(isoprenaline) 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 1 Concentration dependent relaxation by (−)isoprenaline on trachea preparation of rat after contraction by 10−6M (1), 3·10−6 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 (10−6M) and subsequent isoprenaline concentration dependent relaxation at TDI concentrations of 0 (1), 10−4 M (2), 10−5 M (3), and 10−5 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.
metacholine and subsequent isoprenaline line concentration
experiments.

58

-5

55

75

95

Log (isoprenaline)(M)

% Contraction

Fig 3 Absence of any effect of TDI (10⁻⁴ M dissolved in
DMSO) on precontraction of trachea ring induced by 10⁻⁴ 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
by varying metacholine concentrations (fig 1). The
insert in fig 1 shows the concentration response rela-
tion for metacholine. From fig 1 it is clear that when
increasing the precontraction of the trachea ring, the
pD₂ value (logarithm of the isoprenaline concentra-
tion that produces a half maximal relaxation) de-
creases, whereas the maximal relaxation also
decreases.

Figure 2 shows the effect of preincubation and presence
of variuos concentrations of TDI added in
DMSO (fig 2a) or in water (fig 2b) on the relaxation
curves induced by isoprenaline in the tracheal prepara-
tion. Firstly, we observed a dose dependent activation of
the muscarinic response to 1 μM 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 concen-
tration 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 (10⁻⁴ M, 10⁻⁵ M, 10⁻⁴ M) on the
half maximal contracted strip (contraction induced by
1 μM metacholine), nor was any effect detected of TDI
on the isoprenaline induced relaxation of the strip
brought into contraction with 1 μM 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
agonist isoprenaline. Moreover, McKay and Brook,
using frog erythrocytes, showed that direct stimula-
tion 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 bron-
choconstrictive 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 any
effect on the metacholine response in the lung paren-
chymal 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 (fig
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
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 addi-
tion, owing to the chemically reactive properties of
TDI, a wide variety of other functionally important
components mediating metacholine response may be
affected. Irrespective of the ultimate cause of the
increased metacholine responsiveness, it implies that
relaxation by any β-adrenergic agonist demands a
higher equiactive concentration of the agonist. In fig
it is shown that isoprenaline relaxes the rat tracheal
strip preparation previously precontracted by meta-
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carefully their technical are response) control becomes abnormal (diminished mechanisms lungs, the tissue may result from quantitative effect of In vitro to applied the in preparation. Identical experimental conditions used in a preparation determines passive force and precontraction (0.4 g passive force and precontraction with 1 μM 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).

We are greatly indebted to B de Rooy and A Civil for their technical help and to Marliese van Wissen for carefully preparing the manuscript.

Requests for reprints to: Dr P J A Borm, Department of Occupational Medicine, State University of Maastricht, PO Box 616, 6200 MD Maastricht.

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