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Development of an In Vitro Model Assay System for the Evaluation of the Effects of Toxic Chemicals on Human Airways

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13. ABSTRACT *(Maximum 200 words)*
The ability of the anticholinesterase agent soman to contract human bronchi was examined. Soman (1-2 uM) had variable effects on human bronchi that had not been stimulated with an electric field stimulator (EFS). In bronchi continuously stimulated by EFS (0.5 Hz, 1 ms, 12 V), soman produced contractions in all tissues examined (12 preparations from 9 humans). In tissues stimulated by EFS, the beta-adrenoreceptor agonist isoproterenol produced relaxations that were greater in magnitude than the contractions produced by soman. The duration of the isoproterenol induced relaxations was variable. Of 12 preparations studied, 3 showed no reversal of the relaxation within 120 min, 6 showed a slow reversal with a reversal time of 106 ± 6 min and 3 showed rapid reversal with a 50% reversal time of 14 min. In the latter group the duration of the relaxation produced by isoproterenol was doubled (28 ± 2 min) by the M2 muscarinic recptor antagonist AFDX 116 (10 uM). These results show that the isolated human bronchus is a useful model for studying the effects of toxic chemical agents such as soman on the airways. The data obtained with isoproterenol suggest that beta-2 agonists may be useful adjuncts for treating the effects of anticholinesterase agents.

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human bronchi, soman, isoproterenol, AFDX-116

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FOREWORD

This research was carried out under research plan 1-03-15-0000, protocol No. 1-03-87-015-A-443 "Effect of Anticholinesterase Agents on Isolated Human Airway Smooth Muscle," Task Area 875, WBS TechBase, Task NA 1.4, NA 1.6, NA 2.1.1 and NA 2.2.1; JSA requirements S-A-302 and S-A-303. Part of this study was supported by Battelle, Columbus Division, 505 King Avenue, Columbus, OH 43201 under Scientific Services Program Contract No. DAAL03-86-D-0001, Delivery Order No. 1961; TCN 90-088, under the auspices of the US Army Research Office, Research Triangle Park, NC 27709-2211.

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INTRODUCTION

Exposure of personnel to anticholinesterase nerve agents such as soman produces contraction of bronchial smooth muscle, thus increasing airway resistance. Increase in airway resistance leads to wheezing and shortness of breath which in severe cases may be life-threatening (1-5). The contraction of airway smooth muscle by soman and similar agents is due to the accumulation of acetylcholine released from parasympathetic nerves which supply the main neuronal drive in human airways. The accumulation of acetylcholine is due to the inhibition by soman of cholinesterases which would normally inactivate the acetylcholine by hydrolysis.

Because these agents increase the accumulation of acetylcholine at its receptors, the drug of choice in treating nerve agent poisoning has been the muscarinic antagonist atropine (2). Recently, however, investigators have been studying other drugs as possible therapeutic agents. One such class of compounds has been the *beta*-2 adrenergic receptor agonists typified by isoproternol. These drugs, which have long been used in the treatment of respiratory diseases such as asthma, have also been shown to functionally antagonize cholinergic responses (6). Animal models have shown that isoproternol will reverse the contraction produced by soman in both canine (7) and guinea pig (8) airways. Differences between the reversal produced by isoproternol are apparent between canine and guinea pig airways. In canine airways, the isoproternol relaxation was rapidly reversed and the response showed tachyphylaxis in that subsequent addition of isoproternol produced no relaxation (7). In contrast, the relaxations produced by isoproternol were longer lasting in the guinea pig and were reproducible upon further application of isoproternol (8). Few studies, however, have examined the effects of nerve agents in human isolated airways; thus, it is unknown whether the animal studies represent the human condition. This study, therefore, examined the ability of soman to contract human isolated bronchi *in vitro* and the ability of isoproternol to relax soman-contracted airways.

METHODS

Human bronchus.

Macroscopically normal, human lung tissue was obtained from organ donors. The average age of the organ donors was 38±4 years; three were male and six were female. The cause of death was primarily head trauma from motor vehicle accidents or gunshot wounds and from cerebrovascular incidents. The organ donor specimens were placed in Viaspan (Dupont Merck Pharmaceuticals, Wilmington, DE) at 4°C and transferred overnight to the laboratory. On arrival at the laboratory, the specimens were placed in a Krebs' solution gassed with 95% oxygen and 5% carbon dioxide at 4°C. Bronchi (5-10 mm i.d) were trimmed of surrounding parenchyma and blood vessels. The bronchi were cut longitudinally and prepared as transverse strips 4-5 mm wide. The bronchi were suspended between platinum ring electrodes in 10 ml organ baths containing Krebs' solution. The tissues were connected to Grass FT03 force transducers for the measurement of isometric tension which was recorded on a Gould polygraph. The electrodes were connected to a Grass S44 stimulator whose output was passed through a Medlab Stimu-splitter (Fort Collins, CO) for measurement of current across the electrodes and for signal amplification. The current applied in these experiments was 200-300 mA. Preparations were suspended at an initial tension of 2g and washed with fresh buffer every 15 min for a 60 min equilibration period.

After the equilibration period, tissues were either left unstimulated or stimulated continuously (0.5 Hz, 1 ms, 12 V). Soman (1-2 μM) was then added to the baths. After the soman-induced contraction had reached a plateau, isoproternol (1 μM) was added and the tension was observed until it returned to the pre-isoproternol level or for 120 min. At this point barium chloride (30 mM) was added to maximally contract the tissues. In some tissues which showed rapid reversal of the relaxation to isoproternol, the ability of AFDX-116 to potentiate the duration of the isoproternol-induced relaxation was studied. In this set of experiments, two control relaxations to isoproternol were obtained; AFDX-116 (1 μM) was then added 5 min before isoproternol. Once the relaxation to isoproternol was completely reversed, 10 μM AFDX-116 was added 5 min before another addition of isoproternol. When tension had returned to baseline, barium chloride was added to maximally contract the tissues.

Data analysis.

Contractions induced by soman (a) were expressed as a percentage of the maximum contraction produced by barium chloride (c) (Figure 1). Relaxations by isoproternol (b) are expressed as a percentage of the contraction by soman (a). A percentage greater than 100 therefore indicates that the relaxation to isoproternol was greater than the contraction by soman. The duration of the relaxation by isoproternol is calculated as the 50% recovery time, that is the time taken for the relaxation by isoproternol to become half of the maximal relaxation.

Drugs.

All drugs were obtained from the Sigma Chemical Co (St. Louis, MO) and prepared fresh daily.
Soman was obtained from the US Army Chemical Research Development and Engineering Center, Aberdeen Proving Ground, MD 21010-5425.

RESULTS

Figure 1 shows that addition of soman to the tissue chamber produced contracture after a short latency. This is designated as "a." Application of 1 μM isoproterenol produced relaxation of the muscle tension; the magnitude of the isoproterenol-induced relaxation is designated as "b." The maximum contraction obtained with each tissue was determined by the addition of barium chloride (30 mM) at the end of the experiment. This is designated as "c." Contractions produced by barium chloride were always greater than those obtained in the presence of soman alone. Figure 1 shows that isoproterenol relaxed the tension below the baseline, i.e., before addition of soman. In unstimulated human bronchi, soman (1-2 μM) produced either no response (n=3) or a small contractile response (n=3). In those tissues that contracted, the response to soman application averaged 28$\pm$5% of the maximum response produced by barium chloride. The response to barium chloride averaged 1.80$\pm$0.4 g in these tissues. In the three preparations that contracted upon exposure to soman, isoproterenol (1 μM) relaxed the tension below that of the pre-soman tension (Figure 2A). These relaxations were 183$\pm$26% of the tension produced by soman. The 50% recovery time for the isoproterenol-induced relaxation was 77$\pm$21 min in these tissues.

In tissues continuously stimulated by EFS (0.5 Hz, 1 ms, 12 V), 8 out of the 12 preparations showed no contraction, whereas 4 showed a small contraction that subsided over time. The strength of this contraction at its peak averaged 6$\pm$2% of the maximum response produced by barium chloride. Despite the observation that the majority of tissues continuously stimulated failed to show a contraction to EFS, they all subsequently contracted in the presence of soman (Figures 2 & 3), and isoproterenol (1 μM) elicited relaxations in all of these tissues. However, the duration of the isoproterenol relaxation was quite variable. Three types of responses to isoproterenol were noted: (1) relaxation with no reversal for 120 min (n=3) (Figure 2B); (2) relaxation with slow reversal, having a 50% recovery time of 106$\pm$12 min (n=6) (Figure 2C); (3) rapid reversal with 50% recovery time of 14$\pm$1 min (n=3) (Figure 3).

In tissues that did not exhibit reversal of relaxation, soman-induced contractures averaged 29$\pm$7% of the response to barium chloride. The response to barium chloride in these tissues was 0.8$\pm$0.28 g. Relaxations produced by isoproterenol averaged 214$\pm$38% of the soman contractions. In tissues showing slow reversal of relaxation, the contracture induced by soman averaged 39$\pm$7% of the response to barium chloride. The response to barium chloride in these tissues was 1.87$\pm$0.04 g, and isoproterenol produced relaxations averaging 160$\pm$14% of the tension produced by soman. In tissues exhibiting rapid reversal of the isoproterenol-induced relaxation, the contraction produced by soman averaged 39$\pm$5% of the response to barium chloride. The response to barium chloride in these tissues was 1.83$\pm$0.2 g, and isoproterenol produced relaxations averaging 198$\pm$7% of the soman-induced contractions.

The effect of AFDX-116, a muscarinic M_2 receptor subtype antagonist, on the duration of the relaxation produced by isoproterenol was examined in the group exhibiting rapid reversal of the isoproterenol-induced relaxation. A initial application of isoproterenol (1 μM) had a 50% recovery time of 14$\pm$1 min, and a second application of isoproterenol (1 μM) exhibited a 50% recovery time of 13$\pm$1 min . AFDX-116 (1 μM) had little effect on the 50% recovery time for a third application of isoproterenol (15$\pm$2 min), whereas AFDX-116 (10 μM) produced a doubling of the 50% recovery time for a fourth application of isoproterenol (28$\pm$2 min) (Figure 3). In 2 out of the 3 preparations, AFDX-116 (10 μM) produced a small relaxation (8% of that produced by isoproterenol), suggesting that at this concentration AFDX-116 had an effect on both M_3 and M_2 muscarinic receptor subtypes.

DISCUSSION

The results of the present study show that soman is capable of contracting human isolated bronchi. This is in agreement with animal studies where soman has been shown to contract the isolated airways from several species including canine (7), guinea pig (9) and rat (10). The results with unstimulated tissues indicate that the release of acetylcholine in these tissues is variable and is in agreement with studies which have shown that atropine has little effect on intrinsic tone in human airways (11). Soman, however, contracted all the tissues that were continuously stimulated at low frequency (0.5 Hz), even those tissues that showed no contractile response to this stimulation in the absence of soman. It would, therefore, seem prudent that this procedure be used in subsequent studies in human bronchi where the effect of soman and similar agents is to be studied.

The *beta*-2 adrenergic receptor agonist isoproternol was able to relax all the tissues which exhibited contractions by soman. In each case, the relaxation by isoproternol was greater than the contraction by soman, indicating that isoproternol was able to functionally antagonize both the cholinergic response due to soman and the noncholinergic intrinsic tone which is pronounced in human isolated bronchi (11). The relaxations produced by isoproternol were on the whole long-lasting; in 9 out of 12 preparations studied the 50% recovery time was greater than 60 min. In this respect, the response to isoproternol in human airways is more like the guinea pig airways which show slower recovery of the isoproternol relaxant response than do canine airways (7). In 3 out of 12 tissues, the reversal of the isoproternol response was fairly rapid although still slower than that observed in canine airways. In the latter group, the addition of another dose of isoproternol produced a relaxation similar to the first relaxation. Although these findings are similar to those observed in the guinea pig, they differ markedly from the results obtained in the canine airway where the subsequent addition of isoproternol had no effect (7). Also in agreement with the study on guinea pig airways, the M2 muscarinic receptor antagonist AFDX-116 prolonged the relaxation elicited by isoproternol.

The reversal of the isoproternol relaxation may be due to a number of factors:

1. Tachyphylaxis. *Beta*-2 adrenoceptors have been shown to be desensitized by repeated administration of an agonist. The continued presence of isoproternol in the bath may therefore produce internalization of receptors or other events which produce tachyphylaxis. In the present study this is unlikely to be the case, as repeated administration of isoproternol produced similar results.

2. Breakdown of the isoproternol. Isoproternol can be rapidly reduced, leading to loss of activity. This would not explain, however, how the duration of the response to isoproternol is enhanced by AFDX-116.

3. Functional antagonism. Isoproternol produces relaxations of airway smooth muscle by activating adenylate cyclase leading to increased cyclic AMP, whereas stimulation of muscarinic receptors produces the opposite effect and will thus tend to oppose relaxations elicited by isoproternol (12-14). The ability of AFDX-116 to prolong relaxations to isoproternol suggests that M2 receptors may be involved in this functional antagonism. Supporting this argument is the finding of Fernandes *et al.* (15) which showed that isoproternol-induced relaxations of methacholine-induced tone in canine trachea were augmented by AFDX-116.

CONCLUSIONS

The human isolated bronchus serves as a very useful model system to study the effect of nerve agents such as soman. Moreover, *beta*-2 adrenergic agonists may serve as useful adjuncts to conventional atropine therapy in the treatment of nerve agent poisoning, furthermore, the usefulness of *beta*-2 agonists may be enhanced by M2 antagonists. Additional studies should be carried out on human airways to examine further possibilities for treatment of nerve agent poisoning. These might include evaluation of new longer acting *beta*-agonists, calcium channel blockers and potassium channel activators.

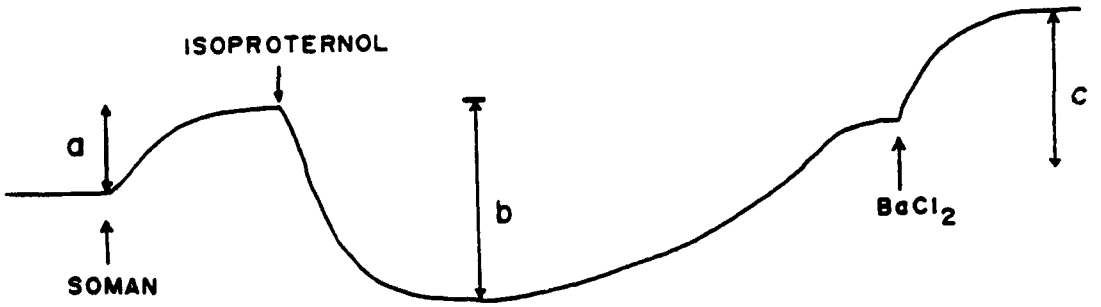

Figure1. Diagram of research protocol. Soman was added to either unstimulated or stimulated (0.5 Hz, 1 ms, 12 V continuously) human bronchus. The contraction by soman is designated "a". Once the contraction by soman has reached a plateau 1 μM isoproternol was added. The relaxation to isoproternol is designated "b". At the end of the experiment tissues were maximally contracted to 30 mM barium chloride. This maximum contraction is designated "c".

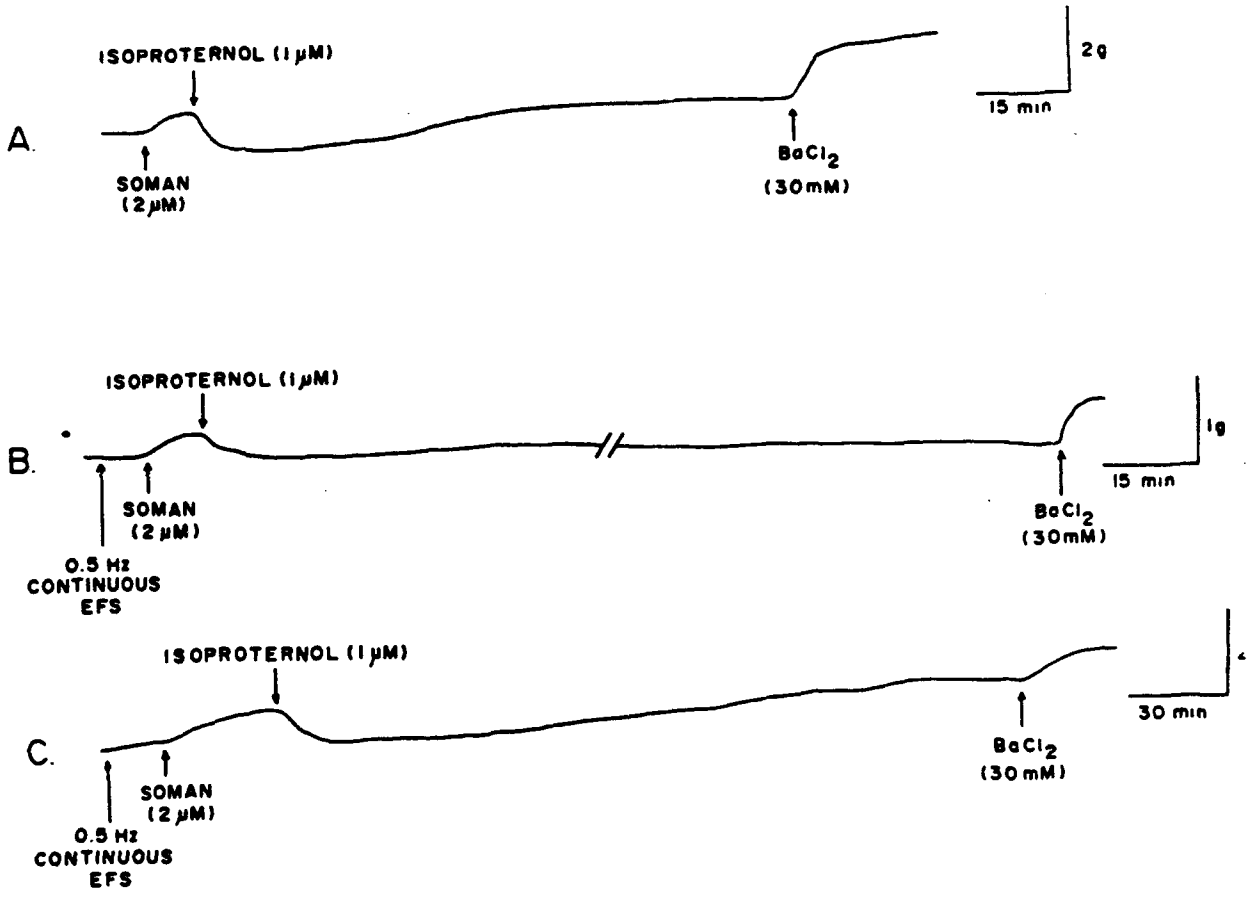

Figure 2. Representative tracings of responses to soman and subsequent relaxations to isoproternol in human isolated bronchi. A) Response to soman in an unstimulated tissue and subsequent relaxation to soman. B). Response to soman in a continuously stimulated tissue (0.5 Hz, 1 ms, 12 V) in which the relaxation to isoproternol was maintained for over 120 min. C). Response to soman in a continuously stimulated tissue (0.5 Hz, 1 ms, 12 V) in which the relaxation to isoproternol returned to baseline.

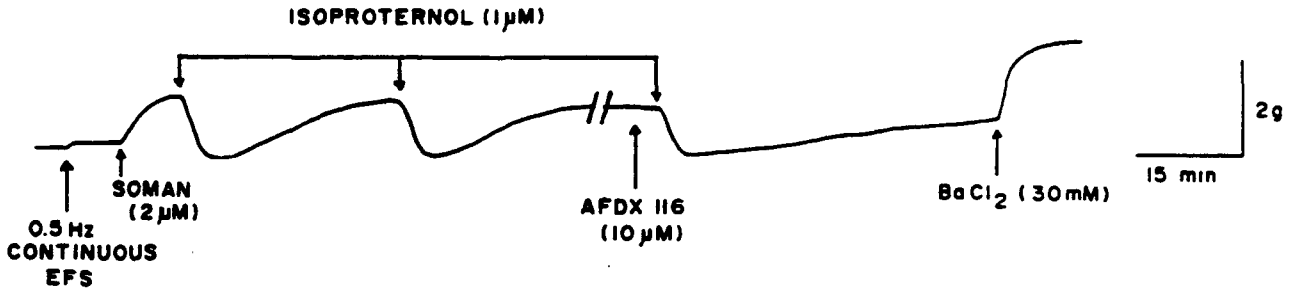

Figure 3. Representative tracing of contraction by soman in a continuously stimulated (0.5 Hz, 1 ms, 12 V) human isolated bronchus in which the relaxation to isoproternol was rapidly reversed. AFDX-116 (10 μM) caused the relaxation to isoproternol to be doubled in duration compared to the two previous control additions of isoproternol.

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