Biochemical Pharmacology, Vol. 35, No. 11, pp. 1793–1799, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
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IN VITRO EFFECTS OF SOMAN ON BRONCHIAL SMOOTH MUSCLE

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(*Received* 11 *October* 1985; *accepted* 21 *November* 1985)

Abstract—The *in vitro* exposure of rat bronchial smooth muscle to the cholinesterase inhibitor soman (O-[1,2,2-trimethylpropyl]-methyl-phosphonofluoridate) potentiated the rapid and concentration dependent increase in the contraction induced by acetylcholine (ACh). There was a substantial increase in the response to ACh when soman was present in concentrations from 10 nM to 1 μM which correspond to a 65–100% inhibition of acetylcholinesterase (AChE). The apparent affinity (pD_2) to ACh increased from 3.7 to 6.7 without any change in intrinsic activity (α) in this concentration interval. In contrast, soman did not alter the apparent affinity or intrinsic activity of carbachol, which supports the suggestion that the effect of soman is entirely due to its anticholinesterase activity. Soman by itself induced contraction which begun at 1–10 nM. This may be explained from its anticholinesterase activity and the subsequent increase in the synaptic concentration of spontaneously released ACh. The effect of soman on inhibition of cholinesterase and carboxylesterases have also been examined. The results demonstrate that low concentrations of soman induces contraction of the airway smooth muscle.

Organophosphorus compounds are widely used as insecticides, some are potential warfare agents and a few are also used in the treatment of human diseases. Their potency is due to the inhibition of acetylcholinesterase, and the subsequent increase in stimulation of cholinergic receptors. Extensive use of organophosphorous compounds is associated with an increased risk of toxicity in man.

The first symptoms to occur during exposure to toxic concentrations of these agents are bronchoconstriction and bronchial secretion [1]. Inhalation of anticholinesterase compounds, e.g. organophosphates constitutes a serious health problem; in this context low concentrations may be life-threatening [2].

The organophosphorus compound soman (O-[1,2,2-trimethylpropyl]-methylphosphonofluoridate) has been used in this study on the effects of anticholinesterases on bronchial smooth muscle *in vitro*. Soman, a potential warfare agent, has previously been shown to inhibit acetylcholinesterase (AChE), pseudocholinesterases (ChE) and carboxylesterases (CarbE) in the respiratory tissue as well as in other peripheral tissues following inhalation [3]. In respiratory tissue CarbEs may have major protective functions, due to their ability to bind several organophosphorus compounds [4, 5]. This may be very important, since the lung is the primary route of entry for these toxic compounds.

We have investigated the effect of soman on bronchial smooth muscle, since the cholinergic portion of the vagus nerve is important in primary motor control of the airways [6]. The study suggests concentrations of soman which are toxic to the bronchi and the results show the correlation between the bronchial contraction and the AChE inhibition.

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MATERIALS AND METHODS

Animals

Male Wistar rats within the weight range 200–300 g were used. The animals were fed standard laboratory diet and given water *ad lib.* The rats were without symptoms of infections in the respiratory system. The rats were examined at the National Institute of Public Health, Oslo.

Chemicals

Soman (O-[1,2,2-trimethylpropyl]-methyl-phosphofluoridate), assessed to be more than 97% pure by nuclear magnetic resonance spectroscopy, was synthesized in this laboratory.

Carbachol was purchased from Sigma Chemical Co. (Poole, U.K.) and acetylcholine chloride and atropine sulphate from Norsk Medisinal Depot, Oslo.

Pharmacological methods

Following decapitation and dissection the left and right primary bronchi were mounted in parallel as circular preparations [7]. The thermostatically controlled organ bath contained Krebs solution (50 ml, 37°) of the following composition (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.6; $MgSO_4$, 1.2; $NaHCO_3$, 24.9; KH_2PO_4, 1.2; Glucose, 11.1. The solution was gassed with 95% O_2 + 5% CO_2 (pH 7.4). For electrical stimulation the bronchi were mounted between platinum electrodes and stimulated by a Grass S88 Stimulator. The preparations were given a preload of 1.0 g and equilibrated for 60 min before the start of the experiments. The contractions were recorded isometrically by Grass Force Displacement Transducers (FT O3C) and monitored on a Grass Polygraph (Model 7) fitted with amplifiers (7 P 1A).

Acetylcholine and carbachol were added by cumulative application, since there was no difference in

apparent affinity (pD_2) when the agonists were added by single or by cumulative application [7].

Soman was added to the *in vitro* preparation and left for 15 min before being removed by washing with the physiological buffer.

Biochemical methods

Determination of acetylcholinesterase and pseudocholinesterase activity. After completion of the physiological experiments (approximately 6 hr) the bronchi were homogenized (2% w/v) in 20 mM sodium phosphate buffer (pH = 7.4) (Ultraturrax Homogeniser; 15 sec, setting 10, ice cold) before enzyme activity assays. Cholinesterase activities were measured by the radiochemical method of Sterri and Fonnum [8]. Acetylcholinesterase activity was measured after inhibition of pseudocholinesterase with ethopropazine [9].

Determination of carboxylesterase activity. Carboxylesterases, which hydrolyze 4-nitrophenylbutyrate, was determined at 30° by a spectrophotometric method [10]. The assay mixture consisted of 0.1 mM sodium phosphate buffer pH = 7.8, 2 mM 4-nitrophenylbutyrate and tissue homogenate in a total volume of 3.0 ml. The stock solution of 4-nitrophenylbutyrate was 0.6 M in acetone. Optical absorbance of 4-nitrophenol at 400 nm was followed in a Varian Cary 118 spectrophotometer, with the assay mixture omitting the tissue homogenate as reference. A molar absorption coefficient of

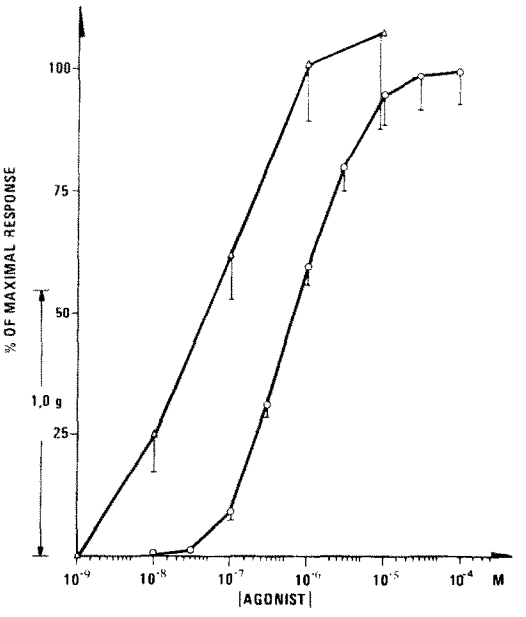

Fig. 1. Concentration–response curves for carbachol (○, N = 35) and soman (△, N = 6–8) on the isolated rat bronchi. The responses (isometric contractions) to carbachol and soman are mean ± S.E.M. of N experiments and plotted in per cent of the maximal response to carbachol prior to exposure to soman in each experiment.

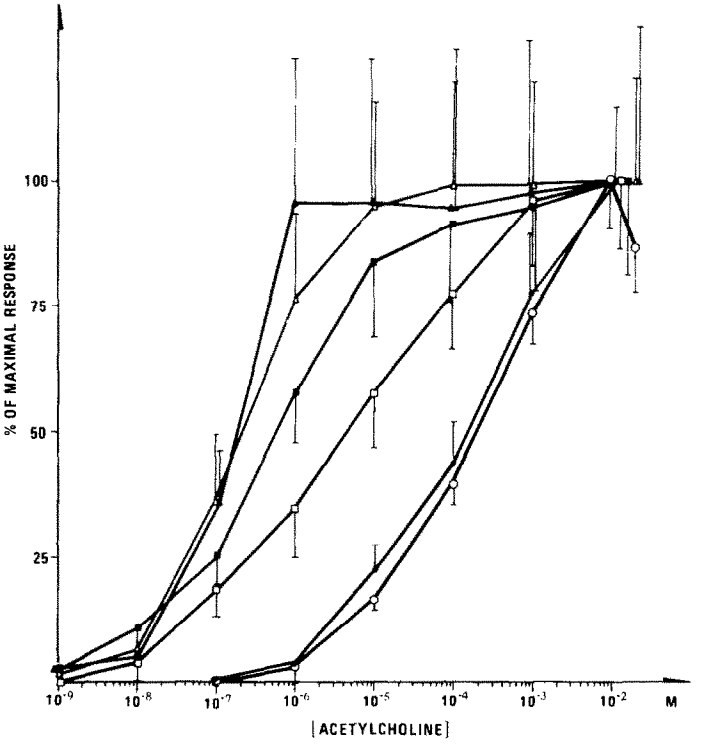

Fig. 2. Concentration response curves for acetylcholine in the presence of soman on the isolated rat bronchi. Control (○, N = 32); soman 1 nM (●, N = 7); soman 10 nM (□, N = 8); soman 100 nM (■, N = 7); soman 1 μM (△, N = 6); soman 10 μM (▲, N = 5). The responses (isometric contractions) are mean ± S.E.M. of N experiments within each group of cumulative application of acetylcholine (in each group the concentration of soman is kept constant).

Table 1. The effect of soman on acetylcholinesterase (AChE), pseudocholinesterase (ChE) and carboxylesterase (CarbE) activities in the rat bronchi

Soman (M)	Enzyme activities (nmol × hr^{-1} × mg $protein^{-1}$ ± S.E.M.			N
	AChE	ChE	CarbE	
0	1264 ± 69	2208 ± 37	52350 ± 3950	2
10^{-9}	942 ± 99 n.s.	3630 ± 369 n.s.	33350 ± 2560*	7
10^{-8}	446 ± 42*	1574 ± 93*	22480 ± 880*	8
10^{-7}	160 ± 21*	550 ± 117*	21330 ± 4360*	7
10^{-6}	13 ± 6*	29 ± 8*	5100 ± 560*	6
10^{-5}	4 ± 4*	46 ± 16*	1990 ± 250*	7

Acetylcholinesterase (AChE), pseudocholinesterase (ChE) and carboxylesterase (CarbE) activities in bronchi after treatment with soman *in vitro*. The bronchi were exposed to soman for 15 min in a physiological buffer prior to recording the contraction induced by cholinergic stimulation. The results are expressed as mean ± S.E.M. and resulted in differences from controls at $^{*}P < 0.01$, $^{n.s.}P > 0.05$.

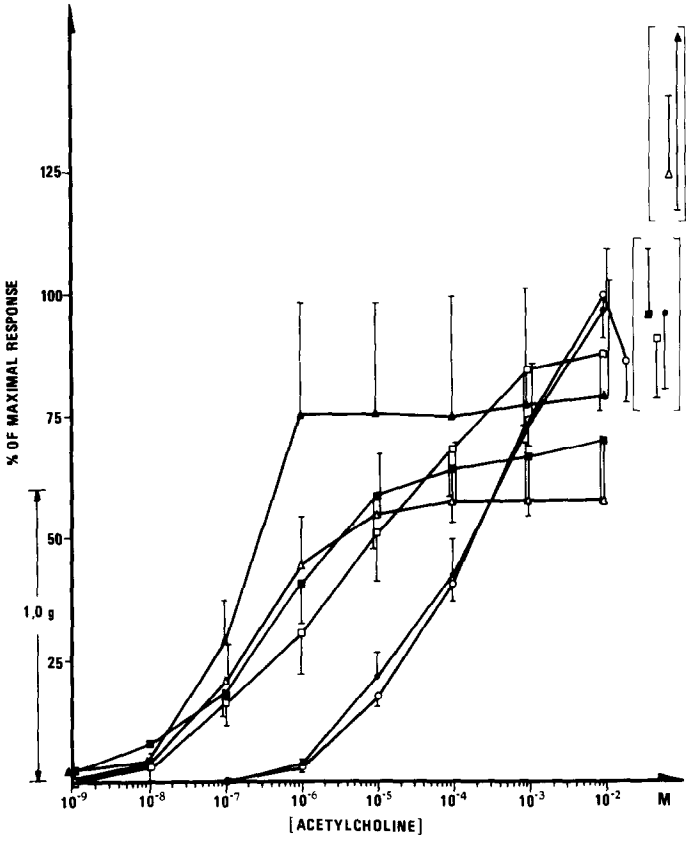

Fig. 3. The effect of soman on cumulative application of acetylcholine on the isolated rat bronchi. Control (○, N = 32); soman 1 nM (●, N = 7); soman 10 nM (□, N = 8); soman 100 nM (■, N = 7); soman 1 μM (△, N = 6); soman 10 μM (▲, N = 5). The responses (isometric contractions) are mean ± S.E.M. of N experiments and plotted in percent of the maximal response to acetylcholine prior to exposure to soman. To the right of the figure in brackets are the intrinsic activities (α') of acetylcholine and soman.

Table 2. The effect of soman on apparent affinity (pD_2) and intrinsic activity (α) of acetylcholine and carbachol in the rat bronchi

Soman (M)	pD_2		α'		α''		N
	Acetylcholine	Carbachol	Acetylcholine	Carbachol	Acetylcholine	Carbachol	
0	3.7 ± 0.1	6.2 ± 0.1	1.00	1.00	1.00	1.00	32
10^{-9}	4.3 ± 0.2*	6.2 ± 0.2 n.s.	1.00 ± 0.18 n.s.	1.00 ± 0.16 n.s.	1.00 ± 0.18 n.s.	1.00 ± 0.16 n.s.	7
10^{-8}	5.3 ± 0.3**	6.5 ± 0.2 n.s.	0.82 ± 0.12 n.s.	0.74 ± 0.11 n.s.	0.88 ± 0.12 n.s.	0.84 ± 0.11 n.s.	8
10^{-7}	6.2 ± 0.1**	6.2 ± 0.2 n.s.	0.71 ± 0.12 n.s.	0.51 ± 0.11**	0.96 ± 0.18 n.s.	0.79 ± 0.16 n.s.	7
10^{-6}	6.6 ± 0.1**	6.2 ± 0.2 n.s.	0.59 ± 0.12 n.s.	0.46 ± 0.11**	1.29 ± 0.18 n.s.	1.16 ± 0.16 n.s.	6
10^{-5}	6.7 ± 0.04**	6.1 ± 0.3 n.s.	0.80 ± 0.20 n.s.	0.60 ± 0.10**	1.50 ± 0.40 n.s.	0.30 ± 0.30 n.s.	7

The intrinsic activity was recorded before and after exposure to soman. α' is the intrinsic activity of the respective agonist after exposure to soman, while α'' is the intrinsic activity of soman and the agonist. The α'- and α''-values are relative to control in the absence of soman, which is given an $\alpha = 1.00$. The results are expressed as mean ± S.E.M. and resulted in differences from control at $^{**}P < 0.01$, $^{*}P < 0.05$, $^{n.s.}P > 0.05$.

17000 $M^{-1}cm^{-1}$ was used [11]. Protein was determined by the method of Lowry *et al.* [12].

Statistics

Means and standard error of the mean (S.E.M.) were calculated for all data. The Student's *t*-test (two-tailed) was applied to the results to determine significant differences between data groups.

RESULTS

The results show that low concentration of soman substantially increased the contraction of bronchial smooth muscle. There was a rapid rise in isometric tension after application of soman (1 nM–10 μM) (Fig. 1) and the increase in muscle contraction correlated well with the inhibition of acetylcholinesterase (AChE) activity (Table 1). There was a concentration dependent inhibition of AChE activity and it was reduced by 65% after exposure to 10 nM soman for 15 min. A complete inhibition of the enzyme resulted following 15 min exposure to 10 μM soman *in vitro*. The intrinsic activity (α) of soman at maximal inhibition of AChE was in the same range as for carbachol (Fig. 1). Carbachol and ACh have previously shown to have the same intrinsic activities in the rat bronchi [7]. Furthermore, soman also markedly potentiated the stimulation of the bronchial smooth muscle by low concentrations of ACh (Fig. 2). The response to increasing concentrations of ACh was considerably enhanced by soman (10 nM–

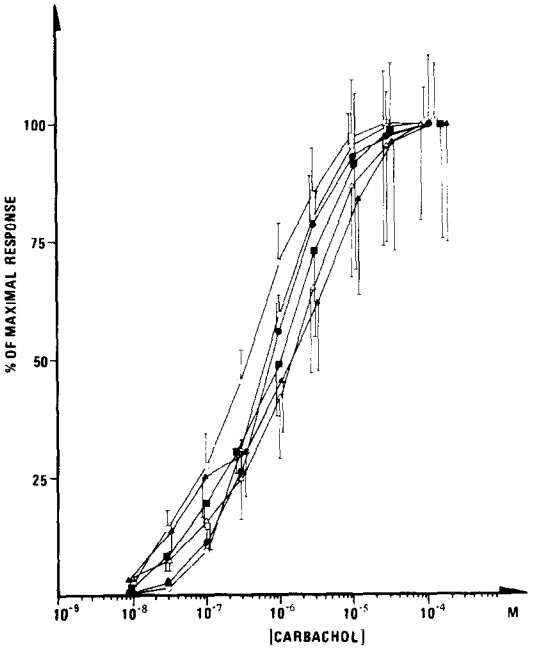

Fig. 4. Concentration response curves for carbachol in the presence of soman on the isolated rat bronchi. Control (○, N = 32); soman 1 nM (●, N = 7); soman 10 nM (□, N = 8); soman 100 nM (■, N = 7); soman 1 μM (△, N = 6); soman 10 μM (▲, N = 7). The responses (isometric contractions) are mean ± S.E.M. of N experiments within each group of cumulative application of carbachol (in each group the concentration of soman is kept constant).

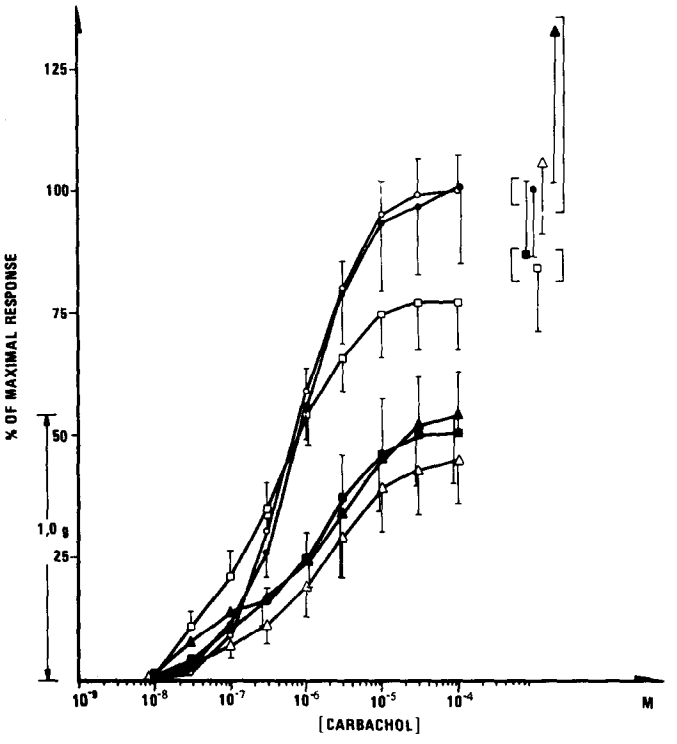

Fig. 5. The effect of soman on cumulative application of carbachol on the isolated rat bronchi. Control (○, N = 32); soman 1 nM (●, N = 7); soman 10 nM (□, N = 8); soman 100 nM (■, N = 7); soman 1 μM (△, N = 6); soman 10 μM (▲, N = 7). The responses (isometric contractions) are mean ± S.E.M. of N experiments and plotted in per cent of the maximal response to carbachol prior to exposure to soman. To the right of the figure in brackets are the intrinsic activities (α'') of carbachol and soman.

1 μM) and the apparent affinity (pD_2) to ACh was increased from 3.7 to 6.7 (Fig. 2, Table 2). This increase in contraction on stimulation by ACh correlates well with the inhibition of AChE (Table 1). On the other hand, soman had no effect upon the contraction induced by maximal stimulation with ACh either when the contraction induced by soman was included (α'') or in its absence (α'), thus the intrinsic activity was approximately constant (Fig. 3, Table 2).

Soman had, as shown in Fig. 4 and Table 2, only limited effects on the apparent affinity (pD_2) of carbachol. There was no significant alteration of the apparent affinity and the total intrinsic activity including the contraction induced by soman (α'') was almost constant in spite of the pronounced inhibition of AChE. Therefore, the intrinsic activities (α'') of ACh and of carbachol including the contraction induced by soman (at a concentration capable of inducing maximal inhibition of AChE) are in the same range. On the other hand, the contraction (α') induced by maximal stimulation with carbachol during inhibition of AChE by soman was reduced (Fig. 5, Table 2), therefore a decrease in intrinsic activity (α') to carbachol was observed. The pD_2-value for carbachol was considerably higher (pD_2 = 6.2) than for ACh (pD_2 = 3.7), even though carbachol and ACh had similar intrinsic activities.

In addition to AChE, soman also substantially inhibited pseudocholinesterase (ChE) and carboxylesterase (CarbE) activities (Table 1). There was a concentration dependent inhibition of ChE and CarbE which was inhibited by 29% and 57% respectively after exposure to 10 nM soman for 15 min. A complete inhibition was obtained after exposure to 10 μM for 15 min.

The electrical field stimulation, which induced release of ACh and subsequent contraction of the bronchial smooth muscle [7], was also enhanced by soman (Fig. 6). There was a large increase in the electrically induced contraction at low concentrations of soman (10 nM). At higher concentrations a smaller enhancement due to electrical stimulation was observed, although the total contraction induced by soman plus electrical stimulation was larger. Soman potentiated the contraction induced by increasing both the frequency and duration of stimulation.

Atropine (7.2 μM) did not only abolish the effect of stimulation with ACh and carbachol, but also inhibited the contraction induced by soman. During AChE inhibition, atropine prevents the spontaneous released ACh in inducing contraction [13].

DISCUSSION

We have shown that soman in low concentrations substantially increased the contraction of rat bronchial smooth muscle. Soman, which is a specific irreversible acetylcholinesterase inhibitor [14]

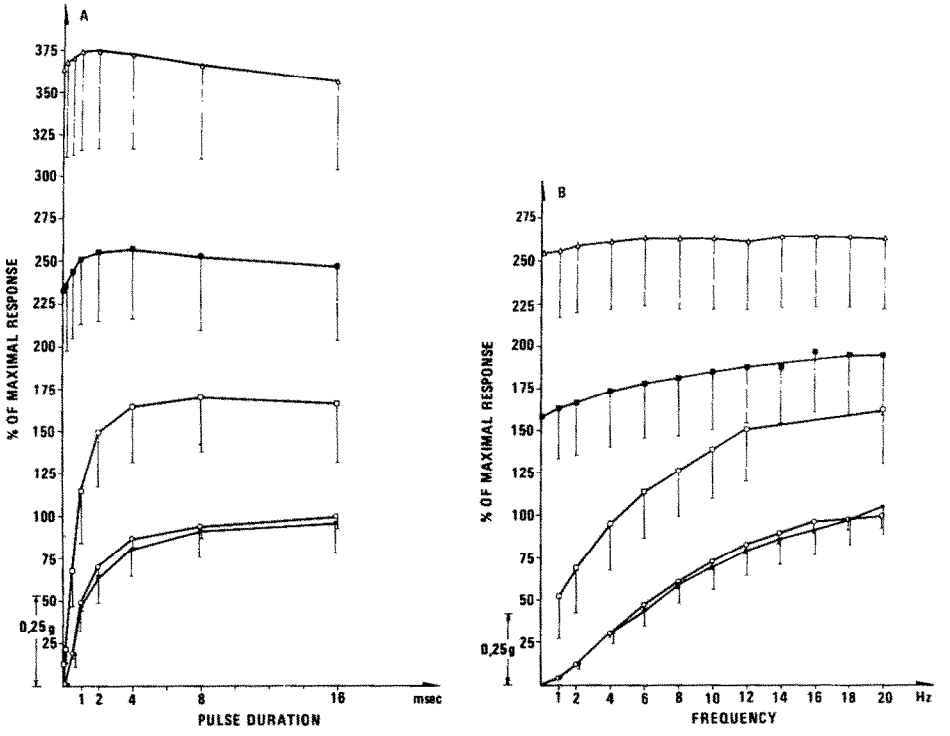

Fig. 6. Effect of electrical stimulation of the isolated rat bronchial smooth muscle: (A) pulse duration (0.1–16 msec) and constant pulse frequency (10 Hz); (B) pulse frequency (1–20 Hz) and constant pulse duration (6 msec). The responses are mean ± S.E.M. of N experiments and plotted in per cent of control stimulation without soman (○, N = 35); soman 1 nM (●, N = 7); soman 10 nM (□, N = 8); soman 100 nM (■, N = 7); soman 1 μM (△, N = 6).

increases the concentration of ACh in the cholinergic synapses and therefore increases the contraction of the muscle. The increase in contraction was rapid and correlated well with the inhibition of AChE activity, which was substantially inhibited at 10 nM of soman (Table 1).

The results show that soman increased the apparent affinity (pD_2) to ACh substantially whereas the apparent affinity to carbachol was unchanged. The effect of soman is therefore probably only due to its anticholinesterase activity. Since a very low concentration of soman (10 nM) increased by some 30% the half maximal contraction of the bronchial smooth muscle induced by stimulation with ACh, even low atmospheric concentrations of this anticholinesterase may play an important role in toxicity. The effect of soman on the smooth muscle is probably only due to inhibition of esterase activity rather than stimulation of the muscle cells *per se*, since the contraction is inhibited by atropine. Hence, there is a close correlation between the tonus of the bronchial smooth muscle and the concentration of the AChE-inhibitor.

In the presence of soman (10 nM–10 μM) an apparent decrease in the intrinsic activity (α') to stimulation with carbachol was observed (Fig. 5 and Table 2). This decrease in contraction was probably due to inhibition of AChE by soman and the subsequent accumulation of spontaneously released ACh followed by the stimulation of muscarinic receptors. The increase in synaptic concentration of endogenously released ACh by soman is clearly shown during electrical stimulation. However, it is difficult to explain the difference in the intrinsic activities (α') between ACh and carbachol since AChE is completely inhibited at 10 μM of soman. On the other hand, the contraction induced by both soman and the agonist (α'') did not exceed the contraction induced by the concentration of agonist alone (i.e. concentration necessary to induce maximal contraction (Table 2)). Therefore, contraction due to stimulation with endogenous ACh is obtained at lower synaptic concentration of ACh, since AChE is inhibited.

Furthermore, the experiments showed (Fig. 6) that soman potentiated the electrically stimulated release of endogenous ACh. Electrical field stimulation has been shown to evoke release of ACh in the rat bronchi [7] and the augmentation of contraction by soman was considerable even at low concentrations, due to inhibition of AChE activity. In the presence of high concentrations of soman (0.1–1.0 μM) a very small increase in contraction was observed, this is due to an elevated and sustained contraction of the muscle. Therefore, during exposure to soman there was a substantial increase in the contraction, and this may be due to the spontaneous release of ACh from the cholinergic nerve terminals in the airway smooth muscle. The results, therefore, illustrate the importance of the cholinergic

nervous system, in maintaining normal tone in the airways smooth muscle, and indicate that exposure to even very low concentrations of soman will induce substantial contraction.

As mentioned previously, a concentration dependent inhibition of AChE and ChE was observed after exposure to soman (between 10 nM and 10 μM). During exposure to soman CarbEs were also substantially inhibited. CarbEs are considered to be detoxification enzymes in the body because they bind certain organophosphates [4, 15–17]. It is therefore of general interest to determine the extent of the inhibition of this group of enzymes during exposure to organophosphorus compounds, since they may reduce the inhibition of AChE.

In conclusion, the experiments have shown that there is a good correlation between the activity of AChE and the strength of smooth muscle contraction as shown in Table 1 and Table 2. Furthermore, the results show that a very low concentration of soman (10 nM) has a substantial effect and increase the contraction of the bronchial smooth muscle induced by ACh.

REFERENCES

1. K. P. Dubois, in *Handbuch der experimentellen Pharmakologie* (Ed. G. B. Koelle), p. 833. Springer-Verlag, Berlin (1963).
2. B. Holmstedt, *Pharmac. Rev.* **11**, 567 (1959).
3. P. Aas, S. H. Sterri, H. P. Hjermstad and F. Fonnum, *Toxic. appl. Pharmac.* **80**, 430 (1985).
4. K. Krisch, in *The enzymes*, Vol. 5 (Ed P. D. Boyer), p. 43, Academic Press, New York (1971).
5. W. N. Aldridge and E. Reiner, *Enzyme Inhibitors as Substrates. Interaction of Esterases with Esters of Organophosphorus and Carbanic Acids.* (Eds. A. Neuberger and E. L. Tatum). North Holland, Amsterdam (1972).
6. J. G. Widdicombe, *Physiol. Rev.* **43**, 1 (1963).
7. P. Aas and K. B. Helle, *Regulatory Peptides* **3**, 405 (1982).
8. S. H. Sterri and F. Fonnum, *Eur. J. Biochem.* **91**, 215 (1978).
9. A. Todrick, *Br. J. Pharmac.* **9**, 76 (1954).
10. S. H. Sterri, B. A. Johnsen and F. Fonnum, *Biochem. Pharmac.* **34**, 2779 (1985).
11. Å. Ljungquist and K. B. Augustinsson, *Eur. J. Biochem.* **23**, 303 (1971).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. F. Fonnum, P. Aas, S. H. Sterri and K. B. Helle, *Fund. Appl. Toxicol.* **4**, 52 (1984).
14. D. B. Coult, D. J. Marsh and G. Read, *Biochem. J.* **98**, 869 (1966).
15. D. K. Meyers, *Biochim. biophys. Acta* **34**, 555 (1959).
16. E. Heymann, in *Enzymatic Basis of Detoxification*, Vol. II (Ed. W. B. Jakoby), p. 291. Academic Press, New York (1980).
17. S. H. Sterri, S. Lyngaas and F. Fonnum, *Acta pharmac. toxicol.* **49**, 8 (1981).