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ACUTE AND SUB-ACUTE INHALATION OF AN ORGANOPHOSPHATE INDUCE ALTERATION OF CHOLINERGIC MUSCARINIC RECEPTORS

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Abstract—Acute and sub-acute inhalation exposure of rats to the organophosphorus compound soman (O-[1,2,2-trimethylpropyl]-methylphosphonofluoridate) reduced the contraction of the bronchial smooth muscle induced by cholinergic stimulation. Acute exposure to 8.51 mg/m^3 of soman for 45 min (total dose of 383 mg·min/m^3) inhibited the acetylcholinesterase (AChE) activity of the bronchial smooth muscle by 85% and reduced the contraction induced by ACh and carbachol by 70% and 80% respectively. In spite of the extensive inhibition of AChE and reduction in the contraction following cholinergic stimulation, there was no alteration of the binding capacity (B_{max}) or the equilibrium dissociation constant (K_d) to [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) in the rat bronchi following such an acute exposure.

After sub-acute exposure (40 hr) to 0.45–0.63 mg/m^3 of soman (total dose of 1080–1519 mg·min/m^3) there was a reduction in AChE-activity of 94% and in the contraction of the bronchial smooth muscle induced by ACh and carbachol of 70%. Furthermore, also a reduction of the binding capacity to [^{3}H]-QNB of approximately 40% was observed. Following exposure to soman by both acute and sub-acute inhalation exposure there was an increase in the apparent affinity (pD_2) to ACh in the bronchial smooth muscle, due to the extensive inhibition of the AChE-activity.

Inhalation of soman also induced a substantial inhibition of the AChE-activity in the lung (86%), but somewhat smaller inhibition in the hippocampus (70%) and almost no inhibition in the neostriatum (19%). Moreover, it was only in the lung where sub-acute exposure to soman produced a reduction of the binding capacity to [^{3}H]-QNB and the reduction was approximately 50%. The results therefore show that after sub-acute inhalation of a relatively low concentration of the AChE-inhibitor soman, alterations in the number of cholinergic receptors are only observed in the peripheral cholinergic nervous system.

The toxic effect of a large number of organophosphorus compounds is due to their inhibition of acetylcholine-esterase (AChE) activity. Some of these compounds are widely used as insecticides but some compounds are also potential warfare chemicals. The toxic signs in mammals after acute intoxication include bronchoconstriction, increased bronchial secretion, salivation and lacrimation, muscle fasciculations, generalized tremor, excessive urination and defecation [1].

Previous experiments have shown that a tolerance to the toxicity of organophosphorus compounds may develop during repetitive exposure to sublethal doses [2, 3]. The typical symptoms of organophosphate intoxication are seen with initial doses, but the signs diminish and lead to behavioral tolerance despite continuous inhibition and low activity of AChE [4]. This increase in toleration to organophosphorus compounds such as soman (*O*-[1,2,2-trimethylpropyl]-methylphosphonofluoridate) and DFP (diisopropylphosphoro-fluoridate) has been shown to occur after subcutaneous injections, and it has been suggested that the phenomenon may result from a decreased sensitivity of muscarinic receptors in response to increased levels of ACh [5, 6].

The behaviour of the muscarinic receptor can be studied by using radioactive ligands which bind specifically to the muscarinic receptor. Reduced binding of [^{3}H]-quinuclidinyl benzilate (QNB) associated with chronically low AChE-activity following exposure by subcutaneous injections of organoposphorus insecticides or DFP has shown to result from a decrease in the binding capacity (B_{max}) and only minor alterations of the dissociation constant (K_d). In the brain, a decrease in the binding capacity has been seen in cortex, hippocampal formation, corpus striatum, superior colliculus, lateral septum and in pons. The density of muscarinic receptors was unchanged in cerebellum, brain stem, thalamus and hypothalamus [7, 8]. In the peripheral nervous system muscarinic receptor subsensitivity in the rat ileum has been observed after chronic subcutaneous DFP treatment [9]. This was, however, not caused by a decreased binding of [^{3}H]-QNB, but was primarily due to an increase in the dissociation constant of [^{3}H]-QNB.

There is no available information in the open literature on studies performed on alterations of the muscarinic receptors in the peripheral or central cholinergic nervous system following acute and long-term exposure by inhalation to an irreversible AChE-inhibitor. Therefore, this study had a primary objective to elaborate whether there was any alterations in the muscarinic cholinergic receptors in the respiratory organ after inhalation of soman. Furthermore, it was of interest to correlate any changes

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in the binding capacity (B_{max}) and the dissociation constant (K_d) of [^{3}H]-QNB both in the peripheral and in the central cholinergic nervous system with the remaining AChE-activity.

MATERIALS AND METHODS

Animals. Male Wistar rats (200–250 g) (from Møllegaard, Copenhagen) were given a standard laboratory diet and water *ad libitum*. The animals were kept in standard laboratory cages, six in each, for 1–2 weeks before the start of the experiments. The animals were kept on sawdust which was replaced daily to ensure that the ammonia concentration was low. The rats had no symptoms of infections in the respiratory tract. They were killed by decapitation and the airways (trachea and primary bronchi), lung, hippocampi and neostriata were dissected out and transferred to the physiological buffer (for composition see Physiological methods and Tissue preparation for binding assay, below). Blood was collected after decapitation.

Chemicals. Acetylcholine chloride, atropine sulphate and papaverine chloride were purchased from Norsk Medisinaldepot in Oslo. Carbachol and bovine serum albumine, fraction V were purchased from Sigma Chemical Co. Poole, U.K. L-[Benzilic-4,4-^{3}H]-quinuclidinyl benzilate (32 Ci/mmol) and [1-^{14}C]-acetylcholine chloride (0.49 mCi/mol) were purchased from New England Nuclear Corporation (Boston, MA). Soman (O-[1,2,2-trimethylpropyl]-methylphosphonofluoridate) assessed to be more than 99% pure by nuclear magnetic resonance spectroscopy, was synthesized in this laboratory. All other chemicals were of analytical laboratory reagent grade.

Physiological methods. The left and right bronchi were mounted in parallel as circular preparations on hooks made from cannulas as previously described by Aas and Helle [10]. The preparations were maintained in a thermostatically controlled organ-bath (37°) containing Krebs buffer to permit measurement of isometric contraction following stimulation. The Krebs buffer had 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). Acetylcholine and carbachol were added to the buffer in cumulative concentrations. During electrical stimulation the bronchi were mounted between platinum electrodes. The tension was recorded isometrically with Grass Force Displacement Transducers (FT-03) and a Grass Polygraph (RPS 7A8A) fitted with amplifiers (7P 122). The preparations were stimulated electrically via a Grass S88 Stimulator.

Tissue preparation for binding assay. The airways (the trachea from larynx and the primary bronchi), lungs and brain were rapidly removed. The airways were dissected free from the lung and placed in 50 mM sodium–potassium phosphate buffer (pH = 7.4, 4°). The hippocampi and neostriata were excised from the remaining brain tissue and left in the buffer at 4° before homogenization in a glass–Teflon homogenizer. Lung and airways were homogenized by a Polytron instrument (setting 10, 20 sec, ice-cold) in sodium–potassium phosphate buffer (pH = 7.4). A 5% homogenate was made of the primary bronchi and trachea, lungs, hippocampi and neostriata. Lung and airway homogenates were filtered through one layer of gauze before centrifugation at 50,000 *g* for 15 min (4°). The pellets were rehomogenized twice in sodium–potassium phosphate buffer and centrifuged at 50,000 *g* for 15 min (4°). The supernatants were discarded. The final pellets were resuspended in sodium–potassium phosphate buffer to the initial volume and stored for less than 60 min at 4° before use in the binding experiments.

Binding assay. Binding assays were performed using a modification of the method of Yamamura and Snyder [11]. Volumes of homogenate, equivalent to 20–40 μg protein for bronchi, 150–200 μg protein for lung and 50–70 μg protein for hippocampus and neostriatum (as determined by the method of Lowry *et al.* [12]) were incubated at 25° for 60 min in sodium–potassium phosphate buffer in a total volume of 500 μl containing 0.004–12.8 nM L-[^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) in the presence and absence of 6 μM atropine. Bound [^{3}H]-QNB was isolated from free by filtration through Titertek filter paper in a Titertek Cell Harvester with continuous washing with 6 ml of sodium–potassium phosphate buffer (pH = 7.4, 25°). The filters were solubilized in Filter-count (Packard Instrument Company) and counted in a Packard Tri Carb liquid scintillation spectrometer (Tri-Carb 300C). Specifically bound radioactivity was calculated by subtracting non-specifically bound [^{3}H]-QNB (CPM in samples incubated with [^{3}H]-QNB plus 6 μM atropine) from total bound [^{3}H]-QNB (CPM in samples incubated with [^{3}H]-QNB in the absence of atropine). All determinations were performed in duplicate or triplicate.

Determination of cholinesterase activity. The cholinesterase activity was determined by the radiochemical method of Sterri and Fonnum [13]. Acetylcholinesterase was measured after inhibition of pseudocholinesterase with etopropazine [14].

Inhalation instrument. Rats were exposed to the acetylcholinesterase-inhibitor soman in an inhalation instrument previously described by Aas *et al.* [15]. The instrument is a dynamic inhalation instrument and the experiments were carried out as whole body exposures to low concentrations of soman. During acute exposure (42–59 min) and sub-acute exposure (2400 min) the rats were exposed to approximately 10 mg/m^3 and 0.6 mg/m^3 respectively.

Analysis of binding data. The binding constants (B_{max} and K_d) were determined from the experimental data by non-linear regression analysis.

Statistics. Means and standard error of the mean (SEM) were calculated for all data. Student's *t*-test was applied to the results to determine significant differences between data groups.

RESULTS

Rats exposed to a low concentration (0.45–0.63 mg/m^3) of soman showed no symptoms of organophosphate intoxication during the 40 hr of exposure. On the other hand, acute exposure (42–59 min) of rats to a higher concentration (8.5–

Table 1. The effect of exposure to soman by inhalation on acetylcholinesterase (AChE) activity and on apparent affinity (pD_2) and maximal response to ACh and carbachol

	AChE		pD_2		Maximal response		
Exposure	nmol·hr^{-1} mg protein^{-1}	%	ACh	Carbachol	ACh	Carbachol	N
Control	789 ± 94	100 ± 11.9	3.8 ± 0.3	6.1 ± 0.05	1.0	1.0	5
Acute	117 ± 38†	14.8 ± 4.8	5.6 ± 0.2†	5.6 ± 0.2*	0.3 ± 0.07†	0.02 ± 0.07†	6
Sub-acute	33 ± 16†	4.2 ± 2.0	5.9 ± 0.1†	5.1 ± 0.2†	0.3 ± 0.05†	0.3 ± 0.05†	8

Rats were exposed to soman by inhalation for 45 min (acute) and for 2400 min (sub-acute) with atmospheric concentrations of soman of 8.51 ± 0.46 mg/m^3 (383 ± 33 mg·min/m^3) (N = 6) and 0.45 ± 0.08 mg/m^3 (1080 ± 281 mg·min/m^3) (N = 8) respectively. The results are mean ± SEM of N experiments. *$P < 0.05$, †$P < 0.01$.

10.4 mg/m^3) induced in several animals increased salivation, dyspnoea, muscle fasciculations, generalized tremor, excessive urination and defecation.

Bronchi from rats exposed to soman by inhalation showed a substantial change in the concentration–response curves and an increase in the apparent affinity (pD_2) to ACh (Table 1). The increase in pD_2 correlated well with the inhibition of acetylcholinesterase (AChE) activity in the rat bronchi which was substantially inhibited by inhalation of soman (Table 1). On the other hand, the apparent affinity to carbachol was slightly decreased relative to control (Table 1) after acute and sub-acute exposure to soman.

The maximal response induced by ACh and carbachol were reduced substantially after both acute (45 min, 400 mg·min/m^3) and sub-acute (2400 min, 1080 mg·min/m^3) exposure to soman relative to control. As shown in Fig. 1 and Table 1 the maximal response induced by ACh was reduced by 70% while the maximal response induced by carbachol was reduced between 70–80% (Table 1). Concomitantly the AChE activity was reduced by 85–95% (Table 1). Neither atropine (7 μM) nor the smooth muscle relaxant papaverine (4 mM) had any effect on an unstimulated bronchial preparation, but they relaxed totally both the ACh or carbachol stimulated preparations (not shown).

In a second series of experiments rats were exposed to similar concentrations of soman by both acute and sub-acute inhalation to compare the physiological effects with receptor binding. In bronchi, lung, hippocampus and neostriatum from these rats [^{3}H]-QNB binding was saturable with increasing concentrations and half maximal binding in the bronchi occurred at approximately 0.3 nM (not shown). The specific binding of [^{3}H]-QNB to bronchi was linear between 10 and 80 μg of protein. The specific binding in lung was linear between 10 and 400 μg of protein and in hippocampus and neostriatum between 10 and 70 μg of protein. At 25° specific [^{3}H]-QNB binding to homogenates of bronchi, lung, hippocampus and neostriatum was half-maximal after 8 min and plateaued by 40 min. The nonspecific [^{3}H]-QNB binding was on the other hand not time-dependent.

The experimental results show that the binding capacity (B_{max}) in rat bronchi was approximately 200 fmol/mg protein which is 14 and 20% of the binding capacity in rat neostriatum (1400 fmol/mg protein) and rat hippocampus (1000 fmol/mg protein) respectively. The B_{max} was on the other hand higher than in the rat lung (30 fmol/mg protein) (Tables 2 and 3). There were no large differences in the dissociation constants (K_d) in the tissues isolated from the rat (Tables 2 and 3).

Following acute exposure (42–59 min) to soman (10.4 mg/m^3) by inhalation with a total dose of 578 mg·min/m^3 there were no changes in the binding capacity (B_{max}) or the dissociation constant (K_d) of [^{3}H]-QNB binding in bronchi (Table 2). The AChE-activity was on the other hand substantially reduced (99%) (Table 4). During acute exposure to soman there was no alteration of the binding constants in the lung, hippocampus or neostriatum (Table 2) in spite of the fact that the AChE-activity was reduced in both lung (98%) and in hippocampus (89%) (Table 4).

On the other hand, following sub-acute exposure (2400 min) to soman (0.63 mg/m^3) with a total dose of 1518 mg·min/m^3 there was a 40% reduction in the binding capacity (B_{max}) to [^{3}H]-QNB in the rat bronchi (Table 3). In spite of a large reduction in the specific binding of [^{3}H]-QNB no changes were observed in the nonspecific binding. During these experiments the AChE-activity was reduced to 5% of control (Table 4). At the same time there was a substantial reduction in the lung AChE-activity and in the [^{3}H]-QNB binding capacity, which were approximately 85 and 50% respectively (Tables 3 and 4). On the contrary no alterations in the binding constants (B_{max} and K_d) were observed in hippocampus or in neostriatum, in agreement with the small inhibition of AChE-activity in these tissues (Tables 3 and 4).

The AChE-activity in erythrocytes and in plasma were also substantially inhibited by inhalation of soman by both acute and subacute exposure. The inhibition of the AChE-activity was somewhat higher during acute exposure as was also seen in bronchi, lung, hippocampus and in neostriatum.

DISCUSSION

Sub-acute inhalation of the AChE-inhibitor soman has been shown to reduce the number of muscarinic receptors in bronchial smooth muscle of the rat as measured by both physiological and biochemical methods. Both the maximal response induced by ACh and carbachol as well as the binding capacity

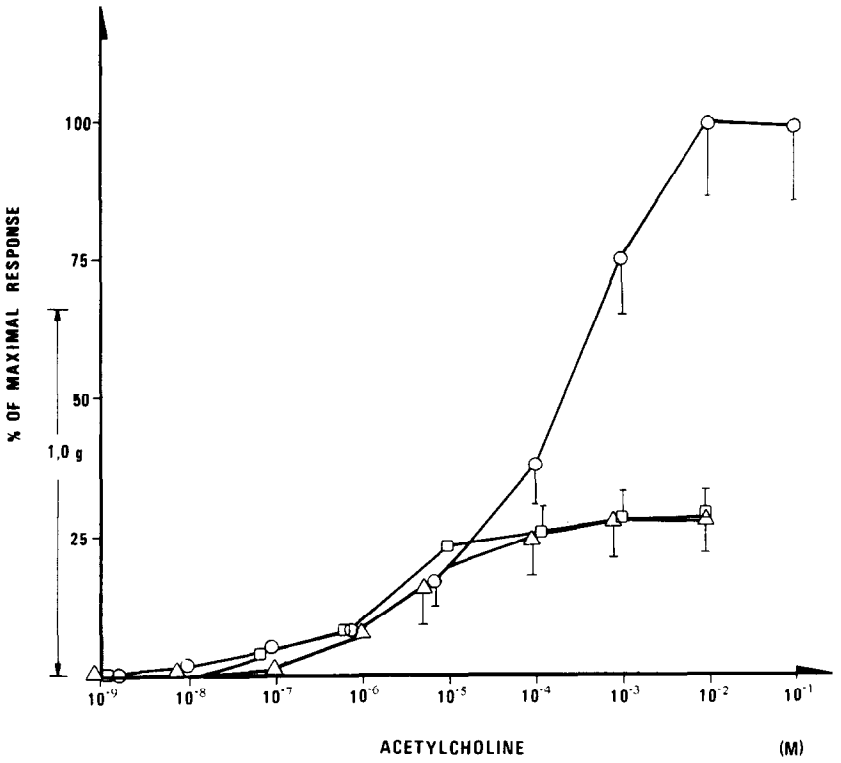

Fig. 1. The effect of soman-inhalation on cumulative application of ACh to bronchial smooth muscle. The responses are mean ± SEM of N experiments and plotted in percent of the maximal response to ACh prior to exposure to soman. Control (○, N = 5); acute exposure (△, 45 min, 8.51 ± 0.46 mg/m^3, 383 ± 33 mg·min/m^3, N = 6); sub-acute exposure (□, 2400 min, 0.45 ± 0.08 mg/m^3, 1080 ± 281 mg·min/m^3, N = 8).

Table 2. [^{3}H]-QNB binding constants in tissues after acute exposure of rats to soman by inhalation

	B_{max} (fmol/mg protein)			K_D (nM)			
Tissue	Control	Experimental		Control	Experimental		N
Bronchi	233.3 ± 28.7	230.3 ± 21.9	ns	0.23 ± 0.03	0.21 ± 0.02	ns	5
Lung	33.1 ± 4.2	28.7 ± 3.2	ns	0.09 ± 0.007	0.12 ± 0.02	ns	5
Hippocampus	966.7 ± 76.0	1071.9 ± 75.9	ns	0.16 ± 0.02	0.13 ± 0.01	ns	5
Neostriatum	1375.8 ± 92.8	1435.3 ± 120.5	ns	0.21 ± 0.03	0.18 ± 0.03	ns	5

Rats were exposed to soman by inhalation for 42–59 min. The atmospheric concentration of soman was 10.4 ± 1.6 mg/m^3 and total dose 578.0 ± 55.7 mg·min/m^3 (N = 5). The binding constants were calculated from saturation binding curves. The results are mean ± SEM of N experiments. ns $P > 0.05$.

Table 3. [^{3}H]-QNB binding constants in tissues after sub-acute exposure of rats to soman by inhalation

	B_{max} (fmol/mg protein)			K_D (nM)			
Tissue	Control	Experimental		Control	Experimental		N
Bronchi	177.8 ± 18.0	107.5 ± 22.8	*	0.16 ± 0.04	0.13 ± 0.03	ns	5
Lung	30.7 ± 1.9	16.2 ± 1.7	†	0.12 ± 0.02	0.16 ± 0.03	ns	6
Hippocampus	1060.1 ± 80.3	801.2 ± 93.4	ns	0.14 ± 0.01	0.14 ± 0.01	ns	6
Neostriatum	1452.6 ± 79.7	1276.5 ± 56.2	ns	0.15 ± 0.01	0.16 ± 0.01	ns	6

Rats were exposed to soman by inhalation for 2400 min. The atmospheric concentration of soman was 0.63 ± 0.01 mg/m^3 and total dose 1518.7 ± 40.0 mg·min/m^3 (N = 6). The binding constants were calculated from saturation binding curves. The results are mean ± SEM of N experiments. ns $P > 0.05$; *$P < 0.05$; †$P < 0.01$.

Table 4. Acetylcholinesterase (AChE) activity in tissues after exposure of rats to soman by inhalation

Exposure	Per cent of control					
	Bronchi	Lung	Hippocampus	Neostriatum	Plasma	Erythrocytes
Acute	1.2 ± 0.5 (5)†	2.1 ± 0.9 (5)†	11.3 ± 8.3 (5)†	47.0 ± 17.9 (5)*	1.1 ± 0.8 (5)†	0.8 ± 0.4 (5)†
Sub-acute	5.1 ± 1.3 (6)†	14.3 ± 3.8 (5)†	30.9 ± 11.6 (6)†	81.5 ± 8.9 (5)ns	16.4 ± 4.7 (6)†	1.1 ± 0.5 (6)†

Rats were exposed to soman by inhalation for 42–59 min (acute) and for 2400 min (sub-acute) with atmospheric concentrations of soman of 10.4 ± 1.6 mg/m^3 (578.0 ± 55.7 mg·min/m^3) (N = 5) and 0.63 ± 0.01 mg·min/m^3 (1518.7 ± 40.0 mg·min/m^3 (N = 6) respectively. The results are mean ± SEM of N experiments. ns $P > 0.05$; *$P < 0.05$; †$P < 0.01$.

(B_{max}) of [^{3}H]-QNB were significantly reduced and the alteration correlated well with the substantial inhibition of the AChE-activity. Concomitantly there was also a correlation between the AChE-inhibition and the reduction in binding capacity in lung after inhalation of soman. On the other hand there was no alteration of the [^{3}H]-QNB binding capacity in hippocampus or in neostriatum, although in hippocampus the AChE-activity was reduced by approximately 70%. In contrast, acute exposure to soman by inhalation did not result in any alteration of [^{3}H]-QNB binding in rat bronchi although the contraction of the bronchial smooth muscle induced by ACh or carbachol stimulation was reduced.

The nervous system has proved to have the ability to adapt to toxic concentration of acetylcholinesterase (AChE)-inhibitors. Several compensatory and recovery mechanisms have been described depending on the duration, level of exposure and type of inhibitor [2, 4, 16]. In previous reports [2, 6, 17] studies have primarily been concerned with the effects of organophosphorus AChE-inhibitors on the central nervous system where administration have been by subcutaneous injections. The present study gives results on changes in receptor binding in both the central and the peripheral cholinergic nervous system following acute and sub-acute exposure to soman by inhalation. Exposure through the respiratory system is the most important route of exposure to these chemicals, although skin absorption may also play an important role.

The bronchial smooth muscle from rats exposed to soman by acute and sub-acute exposure responded to contraction induced by cholinergic stimulation at lower concentrations of ACh than bronchi from control rats. As shown in Table 1, there was a significant increase in the apparent affinity (pD_2) to ACh. This is due to the substantial inhibition (85%) of AChE-activity (Table 1) and is in agreement with a previous report where *in vitro* exposure of rat bronchi to soman (10 nM, AChE-activity = 35% of control) increased the apparent affinity to ACh from 3.7 to 5.3 [18]. On the other hand, a decrease in the apparent affinity to carbachol from 6.1 to 5.6 and 5.1 following acute and sub-acute exposure to soman respectively was observed (Table 1). Previously no alterations in the apparent affinity to carbachol have been observed after *in vitro* exposure [18], which is due to the fact that carbachol is not hydrolysed by AChE. The decrease in the apparent affinity seen after acute and sub-acute exposure to soman by inhalation may be a result of soman interaction with the muscarinic receptor.

Tolerance in the bronchial smooth muscle to the toxic effects produced following inhalation of sublethal concentrations of soman was shown to be due to a down-regulation of muscarinic receptors. The alteration of the number of muscarinic receptors in the bronchi was 40% (Table 3). The data presented are consistent with reported decreases in [^{3}H]-QNB binding in several tissues from mammals subcronically treated with diisopropylphosphoro-fluoridate (DFP) by injections [9, 16]. The results therefore indicate that the decreased responsiveness of the bronchial smooth muscle to cholinergic stimulation is due to AChE-inhibition and a following increased

stimulation of muscarinic receptors and therefore reduced number of receptors. This is not unlike the desensitization which occurs at the neuromuscular junction when a prolonged concentration of ACh is applied [19]. The reduction in the number of muscarinic receptors was, however, large and appeared only after sub-acute exposure (Table 3). This concentration did not induce any symptoms of intoxications, although the AChE-activity was substantially inhibited (Table 4).

The reduction in antagonist [^{3}H]-QNB binding might not be due to displacement by other compounds capable of binding to the same binding-site. Such compounds could be excess ACh resulting from the inhibition of AChE and not removed by the assay wash procedure or soman or it's metabolites. Neither of these explanations seems likely, because acute exposure to soman by inhalation, which inhibited AChE-activity to the same extent as following sub-acute exposure, resulted in no decrease in [^{3}H]-QNB binding (Table 2). The decrease in the number of binding sites must therefore be due to a reduction in AChE-activity and a subsequent enhanced and prolonged stimulation of muscarinic receptors by ACh.

Furthermore, the present experiments correlate alterations in antagonist binding with changes in a physiological response. Following sub-acute exposure to soman also a significant reduction in muscle contraction induced by cholinergic stimulation was observed (Table 1). This result corroborates the hypothesis of reduction in the number of muscarinic receptors in bronchial smooth muscle. The change in the maximal contraction response was, however, substantially larger (approximately 70%) than the change in the binding capacity. This difference might, however, be explained by a reduction in the intracellular cascade of transducing mechanisms between the membrane receptors and the contractile filaments following a decrease in the number of muscarinic receptors. On the other hand, also intra-cellular mechanisms, as for example the ability of muscarinic receptors to functionally couple to intracellularly regulatory proteins, intracellular phosphorylation or calcium gating may be altered. This explanation is supported by the fact that there was no reduction in the number of [^{3}H]-QNB binding sites following acute exposure to soman by inhalation although the AChE-activity and the contraction induced by cholinergic stimulation was substantially reduced (Tables 1 and 2). These results indicate further that down-regulation of muscarinic receptors is much more time dependent in contrast to alteration of intra-cellular mechanisms.

Furthermore, a time-dependent down-regulation of muscarinic receptors was also shown in the rat lung (Tables 2 and 3). Only after sub-acute exposure to soman a decrease in the receptor number was observed, even though the AChE-activity was substantially inhibited both after acute and sub-acute exposure. The results show that not only muscarinic receptors in the bronchial smooth muscle down-regulate due to excess stimulation by ACh after prolonged exposure to an anticholinesterase, but also muscarinic receptors in the lung tissue.

Following exposure to soman by inhalation AChE-activity in plasma and erythrocytes were significantly reduced (Table 4). On the other hand, in brain only hippocampal AChE-activity after acute exposure showed any substantial inhibition, which was approximately 90% (Table 4). Hence, during sub-acute exposure soman might undergo detoxification and therefore does not reach the brain. Previous studies have shown that plasma contain several enzymes which bind soman irreversibly [20, 21], and these may therefore act as scavengers and reduce the free concentration of soman available to the brain. Therefore, during exposure to low concentrations of soman by inhalation only changes in the peripheral nervous system may occur, leaving the central nervous system relatively unaffected.

In conclusion, the results have shown that sub-acute exposure by inhalation to a relatively low concentration of the organophosphate soman giving no toxic symptoms induce down-regulation of muscarinic receptors in the rat bronchi and in the lung.

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