Br. J. Pharmac. (1983), **78**, 579–589

Therapy of organophosphate poisoning: the marmoset as a model for man

H.P.M. van Helden, H.J. van der Wiel & O.L. Wolthuis

Medical Biological Laboratory TNO, P.O. Box 45, 2280 AA Rijswijk, The Netherlands

1 The ability of various bis-pyridinium oximes to restore organophosphate-inhibited neuromuscular transmission *in vitro* was compared in human intercostal and marmoset diaphragm muscles.

2 HI-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-4′-carbamoyl-pyridinium-1′-methyl ether dichloride monohydrate) appeared very effective against VX (O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate) and sarin in both muscles, whereas obidoxim was quite effective against tabun.

3 Against soman, HI-6, HS-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-3′- carbamoyl-pyridinium-1′-methyl ether dichloride dihydrate) and obidoxim had little effect in the human muscle and only slight activity in the marmoset muscle; HGG-12 (2-hydroxyiminomethyl-pyridinium-1-methyl-3′-phenylcarbonyl-pyridinium-1′-methyl ether dichloride) and benzyl-P2A (1-benzyl-2-hydroxyiminomethyl-pyridinium methanesulphonate) were ineffective.

4 Anaesthetized, atropinized marmosets were poisoned with soman ($4 \times LD_{50}$, i.v.) and subsequently treated with HI-6, HS-6 or HGG-12. Only HI-6 and HS-6 were marginally effective in restoring respiration and neuromuscular transmission.

5 Marmoset muscle is a reasonable model for human muscle for the study of organophosphate poisoning and therapy.

Introduction

The bis-pyridinium oximes HI-6 (2 - hydroxyiminomethyl - pyridinium - 1 methyl - 4′ - carbamoyl - pyridinium - 1′ - methyl ether dichloride monohydrate) and HS-6 (2 - hydroxyiminomethyl - pyridinium - 1 - methyl - 3′ - carbamoyl - pyridinium - 1′ - methyl ether dichloride dihydrate) appeared to be therapeutically active in soman-poisoned mice and very effectively restored soman-inhibited neuromuscular transmission in rats *in vivo* as well as *in vitro* (Kepner & Wolthuis, 1978). HI-6 clearly was the most effective of the two (Wolthuis & Kepner, 1978). A comparison of the results from separate preliminary *in vitro* studies demonstrated considerable species differences between rats and guinea-pigs with respect to the efficacy of these oximes against soman poisoning. This observation led Wolthuis, Vanwersch & Van der Wiel (1981) and Smith, Van der Wiel & Wolthuis (1981) to investigate HI-6, HS-6 and HGG-52 in soman-poisoned isolated external intercostal muscles from humans, dogs, guinea-pigs and rats, and in diaphragm muscle from guinea-pigs and rats. Their results showed that the potency of these oximes for inducing recovery of neuromuscular transmission in soman-poisoned human intercostal muscles was negligible. In a similar *in vitro* study, Smith & Wolthuis (1983) investigated the efficacy of HI-6 in restoring neuromuscular function in soman-poisoned respiratory muscles of the rhesus monkey and found that HI-6 had only marginal reversing effects on neuromuscular blockade of the diaphragm and the intercostal muscles.

The established species differences in the therapeutic efficacy of oximes in soman-poisoned respiratory muscles created the need for a better animal model for man. From an evolutionary point of view the marmoset or the rhesus monkey would perhaps be suitable. Marmosets were chosen for the present study since they were more readily available than rhesus monkeys.

The main purpose of the present study was to compare quantitatively the efficacy of several mono- and bis-pyridinium oximes in soman-poisoned human and marmoset muscle preparations. For the sake of comparison, some of these oximes were also tested after inhibition with some other organophosphate cholinesterase inhibitors. Finally, the efficacy of three bis-pyridinium oximes was tested *in vivo* in anaesthetized, atropinized marmosets poisoned with

soman. The latter experiments were necessary because soman has a predominant central effect in the rat (Meeter & Wolthuis, 1968; Wolthuis, Berends & Meeter, 1981) and also in the guinea-pig (Adams, Yamamura & O'Leary, 1976). Since the oximes induced hypothermia in the rat (Meeter & Wolthuis, 1968; Meeter, Wolthuis & Van Benthem, 1971) indicating that the bis-pyridinium oximes HI-6 and HS-6 penetrate into the central nervous system in effective amounts (Wolthuis *et al.*, 1981), it was necessary to establish whether soman also caused predominant central inhibition in the marmoset and whether bis-pyridinium oximes could protect against soman poisoning *in vivo*.

Methods

Human intercostal muscles

Biopsies of the external intercostal muscle were obtained from thirteen patients, 11 males and 2 females. The mean age of 11 patients (including one female) was 62.7 years with the youngest being 55 and the oldest 71 years. One female was 45 and one male was 17 years. The diagnosis in 7 of the patients was lung malignancy, in one it was tuberculosis, in one pneumothorax, in one Wegener syndrome and in three patients a biopsy of lung tissue had to be taken. All patients gave their consent to the muscle biopsy being removed and were treated at the Department of Thoracic Surgery (Director: Prof. A.G. Brom) of Leiden University or at the Thorax Centre (Director: Prof. J. Nauta) of the Dijkzigt hospital in Rotterdam. Anaesthesia was induced with either: thiopentone, *n*-flurane or halothane in combination with N_2O/O_2 mixtures. The muscle relaxants used were succinylcholine during intubation and pancuronium during the rest of the operation.

Marmoset monkeys (Callithrix jacchus)

The body weights of the marmosets used were between 150 and 350 g; they had been bred and raised in the Primate Centre of the REPGO-institutes of TNO at Rijswijk, The Netherlands. Adult males and females were used. Anaesthesia was induced by intramuscular injection of 30 mg ketamine per animal and maintained with sodium pentobarbitone in a dose of 3 mg/animal i.p., hourly.

Preparation and stimulation of muscle preparations in vitro

The human and marmoset muscle strips were prepared according to the technique described by Wolthuis *et al.* (1981). All muscle preparations were incubated in Krebs-Ringer bicarbonate buffer containing 16.6 mM glucose gassed with 95% O_2 and 5% CO_2, having a pH of 7.4 and a temperature of 37°C.

The preparations were stimulated indirectly via bipolar platinum field stimulation electrodes (Wolthuis *et al.*, 1981). The stimulus parameters used during the experiments were adjusted to produce supramaximal indirect stimulation of the preparations. These stimulus parameters were determined in separate experiments from current strength/pulse duration curves for the threshold of twitch response in the absence and presence of a high dose of tubocurarine (30 μM).

Muscle contractions were measured isometrically with a linear displacement transducer (EMI, type SE 371/1.0) and recorded by a Hewlett-Packard recorder (type 17401 A).

Procedure for in vitro *experiments*

The ability of the muscle strips to sustain a tetanic contraction was tested with four 3 s periods of stimulation, at 30 s intervals and at frequencies of 12.5, 25, 50 and 100 Hz respectively (Wolthuis *et al.*, 1981). Following control tetani (test A, see Figure 1), soman (1 μM) was added to the bath and removed 5 min later by washing. Tetanic contractions were then measured (test B) to confirm neuromuscular blockade. Subsequently, oxime was added to the bath. The standard oxime dose was 1.5 mM; for HGG-12 (2 - hydroxyiminomethyl - pyridinium - 1 - methyl - 3' - phenylcarbonyl - pyridinium - 1' - methyl ether dichloride), HI-6 and HS-6 lower doses were also used. Tetanic contractions were retested 10 min later in the presence of the oxime (test C) and once again 5 min after removal of the oxime (test D). Then, a second dose of soman (2 μM) was added to the tissue bath and tetanic responses were tested 5 min later (test E) after wash out of the cholinesterase inhibitor. Between the different tests single twitch responses (0.1 Hz) were recorded. At the end of each experiment tubocurarine was added to the bath to check whether stimulation had been indirect. Cholinesterase inhibitors other than soman were used in the following doses: VX (O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate), 0.75 μM; sarin, 2.85 μM; tabun, 4.93 μM. In all cases a double dose was administered after test D. Control experiments for oxime-efficacy were carried out substituting saline for the oxime. To confirm that no deterioration of the preparations occurred during the testing period, experiments were carried out in which saline was substituted for the various drugs.

Analysis of in vitro *results*

Neuromuscular recovery after organophosphate

poisoning was calculated after a 'blind' assessment of the degree to which each of the four tetani were sustained on a 5 points scale, each point being equal to approximately 20% of neuromuscular transmission. Each control tetanus (in test A) received 5 points, equal to 100% of neuromuscular transmission. The percentages for all four tetanic contractions within a test were added and divided by 4, giving the overall percentage neuromuscular transmission.

Three aspects of neuromuscular recovery were analysed as follows: (1) recovery due to direct oxime action which was estimated by subtracting the percentage neuromuscular transmission in test D (after wash out of oxime) from that observed in test C (oxime present in the bath), i.e. C% − D%; (2) recovery attributed to oxime-induced reactivation of acetylcholinesterase (AChE), estimated by subtracting percentage neuromuscular transmission in test E (after reinhibition by a second dose of the organophosphate) from that in test D, i.e. D% − E%; (3) persistence of neuromuscular recovery notwithstanding full AChE inhibition, i.e. E%, defined as adaptation. According to Meeter & Wolthuis (1968) and Meeter (1969), this adaptation is the result of desensitization of the endplates following prolonged exposure to high levels of acetylcholine.

Statistical analysis was performed using the test of Bartlett (1937) for homogeneity of variances and the Bonferroni-*t* test in combination with the Newman Keuls test (Miller, 1969) for simultaneous comparison of the values C, C% − D%, D% − E%, and E%. In all cases where the term significant is used, $P < 0.05$ (two tailed).

Procedures for in vivo *experiments*

The following parameters were used which reflect the degree of soman intoxication and the recovery after oxime treatment: (1) the percentage of neuromuscular transmission of the indirectly stimulated gastrocnemius-soleus muscles, tested and estimated in the same way as described for the *in vitro* experiments; (2) the respiratory minute volume; (3) the arterial blood pressure; (4) ECG and (5) the heart rate. Technical details were similar to those described previously by Meeter & Wolthuis (1968). Briefly, each animal was provided with: ECG lead II electrodes, stimulation electrodes around the sciatic nerve, a carotid cannula to measure blood pressure, a cannula in the femoral vein for the intravenous injection of drugs, an intraperitoneal cannula for supplying anaesthesia, and a tracheal cannula for artificial ventilation and measurement of the respiratory minute volume. During the experiment the core temperature was kept at 37°C by means of a thermistor controlling a heating lamp ventrally and a heating element dorsally. An additional thermistor, inserted between the hindleg muscles, controlled a separate heating lamp which stabilized the leg temperature at 37°C.

Each animal was treated and tested by the following procedure which is presented schematically in the results (Figure 7). After surgery the animal was heparinized (80 iu per animal). After recording the control tetanic responses (test A), the animal was injected with atropine sulphate (10 mg/kg i.p.), immediately followed by soman (32 μg/kg i.v.) administration. Five min after soman administration, an oxime was given intravenously and 10 min later the tetanic responses were recorded again (test B). Neuromuscular transmission was retested 15 min later (test C), immediately followed by a second oxime injection. Tetanic responses were tested twice during a subsequent period of 30 min (test D and E) which was followed by the administration of a second dose of atropine (10 mg/kg i.p.) and soman (32 μg/kg i.v.) to test which part of the recovery observed was attributable to enzyme reactivation and which part to adaptation (test F). Between the different tests for neuromuscular transmission the sciatic nerve was stimulated at a frequency of 0.1 Hz. Preceding each test for neuromuscular transmission, blood pressure, ECG and heart rate were recorded.

Eighteen adult male and female marmosets weighing 150–350 g were used. To limit the number of animals an LD_{50} value for soman was not determined. Dirnhuber, French, Green, Leadbeater & Stratton (1979) reported a subcutaneous LD_{50} of 8 μg/kg. The intravenous dose given in the present experiments (32 μg/kg) certainly amounts to at least $4 \times LD_{50}$. The doses of the oximes used were 150 or 75 μmol/kg i.v. For the various oximes used the dose of 150 μmol/kg corresponds with: 57 mg/kg HI-6, 59 mg/kg HS-6, 71 mg/kg HGG-12. The 18 animals were divided into a control group and three oxime-treated groups: a HS-6 group, a HI-6 and a HGG-12 group. These groups consisted of 4, 5, 5, and 4 animals, respectively. From the HS-6 and the HI-6 group, 3 animals were treated with 150 μmol/kg and 2 animals with 75 μmol/kg of the oxime. From the HGG-12 group, 2 animals were treated with the higher dose and 2 with the lower dose of the oxime. The control animals were injected with soman but saline replaced the oxime.

Drugs and chemicals

Soman (O-pinacolyl - methylphosphonylfluoridate); sarin (O - isopropylmethyl - phosphonofluoridate); tabun (O - ethyl - *N*, *N* - dimetyl phosphoramidocyanidate); VX (O - ethyl S - 2 - diisopropylaminoethyl methylphosphonothioate), as well as HS-6 (2 - hydroxyiminomethyl - pryidinium - 1 - methyl - 3′ - carbamoyl - pyridinium - 1′ - methyl

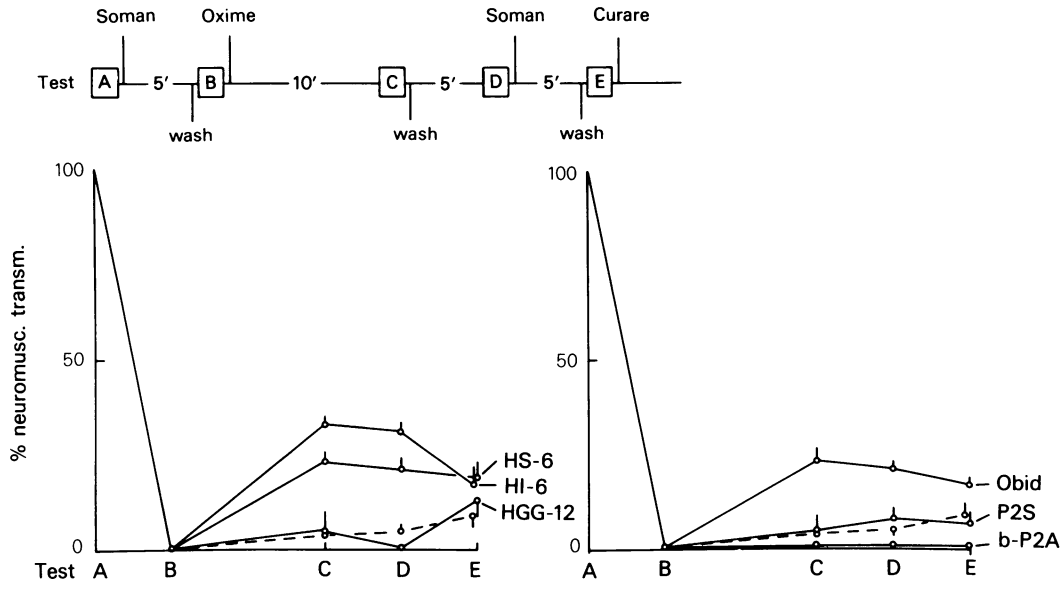

Figure 1 Neuromuscular transmission in human intercostal muscle preparations, expressed as percentages of control values. The effects of various oximes (1.5 mM) were tested after blockade by soman (1 μM). The procedures are schematically depicted at the top of the figure. Test A: control series of tetanic contractions (= 100% neuromusc. transm.); test B: test for completeness of neuromuscular block; test C: recovery in the presence of oxime; test D: recovery of neuromuscular transmission after washout of the oxime; test E: recovery remaining after a second dose of soman (2 μM). At the end curare was given to check whether stimulation had been indirect. Control experiments were carried out in which saline was substituted for the oxime (broken lines). Each value for neuromuscular transmission is the mean of 5 preparations with s.e. mean indicated by vertical lines. Note that HI-6, HS-6 and obidoxim (obid) are only slightly effective in restoring neuromuscular transmission, whereas HGG-12, P2S and benzyl-P2A (b-P2A) are ineffective.

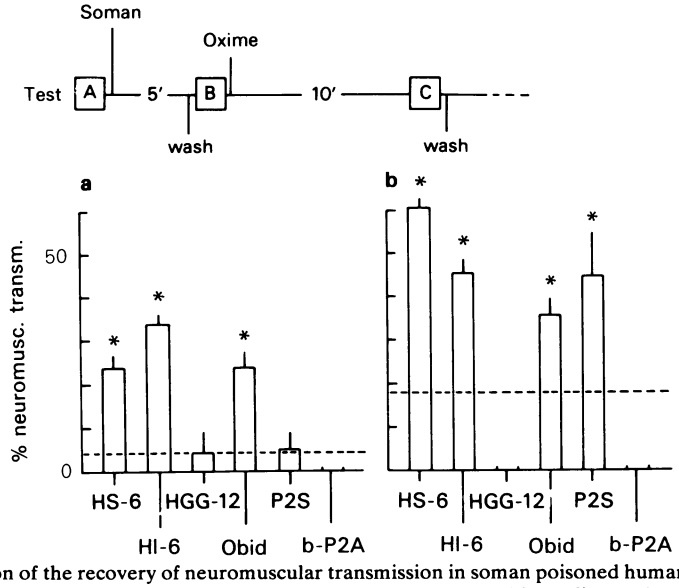

Figure 2 Comparison of the recovery of neuromuscular transmission in soman poisoned human (a) and marmoset (b) muscle preparations in the presence of oxime. These values were collected from Figures 1 and 5. Control values for neuromuscular transmission in test C, i.e. without oxime treatment, are indicated by broken lines. Note that for both species, the effects of HS-6, HI-6 and obidoxim (Obid) are significantly different ($P < 0.05$) from the control value (asterisks) and that P2S is only effective in the marmoset preparation.

ether dichloride dihydrate), HI-6 (2 - hydroxyiminomethyl - pyridinium - 1 - methyl - 4' - carbamoyl - pyridinium - 1' - methyl ether dichloride monohydrate) and benzyl - P2A (1 - benzyl - 2 - hydroxyiminomethyl - pyridinium methanesulphonate) were synthesized by Dr. H.P. Benschop and C. de Borst of the Chemical Research Department of the Prins Maurits Laboratory TNO. The chemical purity of the synthesized organophosphates was: 97.5–98. 5%. The oximes had a satisfactory elementary analysis. According to h.p.l.c.-analysis (Benschop, Konings, Kossen & Ligtenstein, 1981) the purity of the oximes was >98%. HGG-12 (2 - hydroxyiminomethyl - pyridinium - 1 - methyl - 3' - phenylcarbonyl - pyridinium - 1' - methyl ether dichloride), containing 1.5 mol of ethanol per mol of HGG-12, and toxogonin (1,1 - [oxybis (methylene)] bis - [4 - (hydroxyimino)methyl]pyridinium dichloride) were kindly donated by Merck, Germany. P2S (2 - hydroxyiminomethyl - 1 - methyl - pyridinium methanesulphonate) was obtained from Aldrich Europe N.V., Beerse, Belgium. (+)-Tubocurarine hydrochloride was obtained from Asta-Werke AG, Bielefeld, Germany. Atropine sulphate and sodium barbitone (Veronal) were obtained from Brocades N.V., Amsterdam, and sodium hexobarbitone (Evipan) from Bayer AG, Leverkusen, Germany, and ketamine (Vetalar) from Parke-Davis, London. Heparin was obtained from Bipharma B.V., Amsterdam, and sodium pentobarbitone (Nembutal) from S.A. Abbott N.V., Amsterdam.

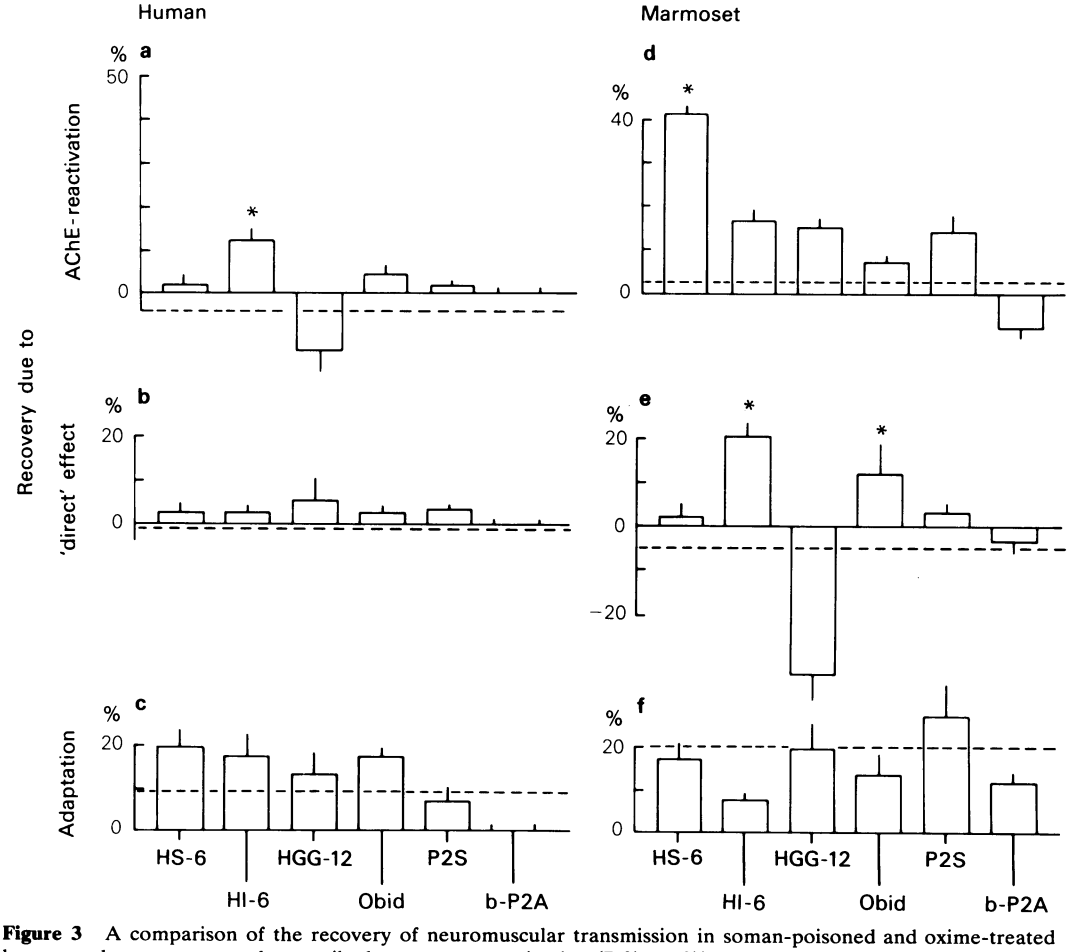

Figure 3 A comparison of the recovery of neuromuscular transmission in soman-poisoned and oxime-treated human and marmoset muscles, ascribed to enzyme reactivation (D% – E%), direct oxime effect (C% – D%) and adaptation (E%). These values are calculated from the results shown in Figures 1 and 5. Control values are indicated by broken lines. In the human muscle (left column) the only significant oxime-effect was the HI-6-induced recovery ascribed to AChE-reactivation (a). In the marmoset muscles the AChE-reactivating effect of HS-6 was significantly higher than the control value (d), whereas HI-6 and obidoxim caused significant 'direct' oxime effects (e). All values are means, s.e.means indicated by vertical lines.

Results

In vitro *experiments*

Human muscle The overall recovery of the neuromuscular transmission obtained with the various oximes after soman-poisoning was small. HI-6 produced the best recovery (Figure 1). HS-6 and obidoxim had equal and small restorative effects on neuromuscular transmission. The recovery after HGG-12, P2S and benzyl-P2A treatment was not significantly different from control values. Lower concentrations of HGG-12 (0.375, 0.150, 0.05 mM) did not result in significantly more recovery of the neuromuscular transmission, neither did treatment with a lower dose of HI-6 or HS-6 (0.75 mM). After treatment with HS-6, HI-6 or obidoxim, the recovery of neuromuscular transmission in soman poisoned muscles in test C, was significantly higher than its control value (Figure 2). From Figure 3 it appears that only after HI-6 the recovery attributable to enzyme reactivation was significantly higher than the values from control preparations. In the human muscle none of the other oximes caused any significant recovery due to either a direct effect, an enzyme reactivation or adaptation.

However, after exposure to sarin or VX, excellent overall recovery could be obtained with HI-6 (Figure 4). Obidoxim was effective against tabun (Figure 4). Analysis of the results showed this recovery to be due to enzyme reactivation (D% − E%, Figure 4) and adaptation (E%) in the case of VX or sarin and only due to enzyme reactivation in experiments with tabun.

Marmoset muscle In the present study the neuromuscular transmission in diaphragm muscles (marmoset) was compared with that in intercostal muscles (human) and hindleg muscles (marmoset). In preceding experiments it had been determined that in the marmoset after soman poisoning no differences existed in the course of the recovery of the neuromuscular transmission in the three types of muscle.

The neuromuscular recovery obtained with oxime treatment after soman poisoning was on the whole somewhat higher than that found in corresponding experiments with the human preparations (Figure 5). In contrast with the results obtained with the human preparations, HS-6 was more effective than HI-6, P2S more than obidoxim. As in human muscles, administration of lower doses of HGG-12 (0.75 and 0.375 mM) did not result in greater recovery of the neuromuscular transmission. With HS-6, HI-6, obidoxim and P2S the recovery in the marmoset

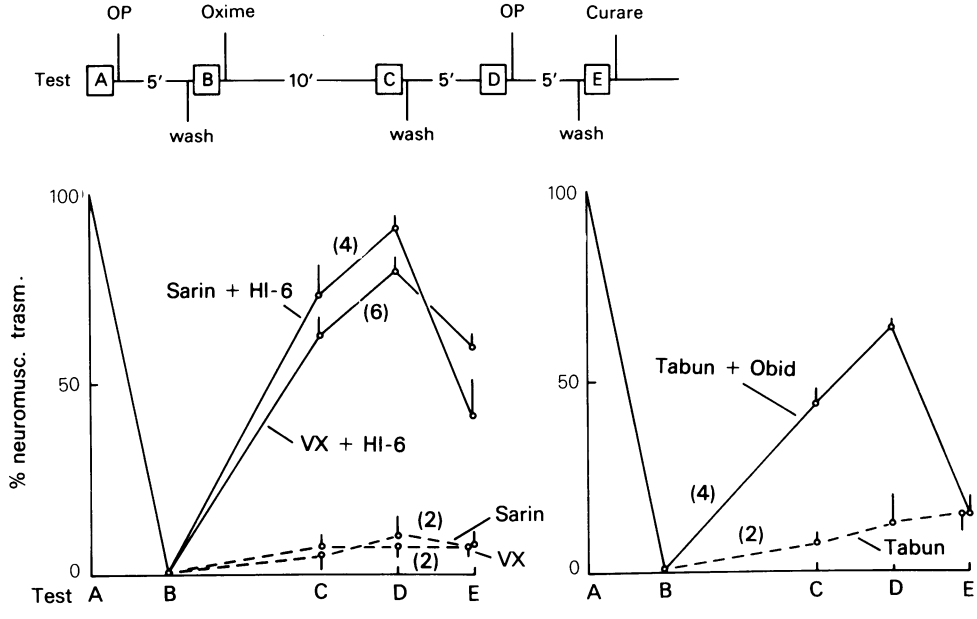

Figure 4 Neuromuscular transmission in organophosphate (OP) poisoned human muscle preparations after oxime treatment. The efficacy of HI-6 (1.5 mM) was tested after sarin (2.85 μM) and after VX (0.75 μM) and the effects of obidoxim (Obid, 1.5 mM) was tested after tabun (4.93 μM). For further details see Figure 1. Control values from preparations without oxime treatment are indicated by broken lines. Each value for neuromuscular transmission is the mean of the number of preparations in parentheses; s.e.means shown by vertical lines. Note that in the human muscle HI-6 is highly effective in restoring neuromuscular transmission after sarin or VX poisoning and that obidoxim is effective after tabun.

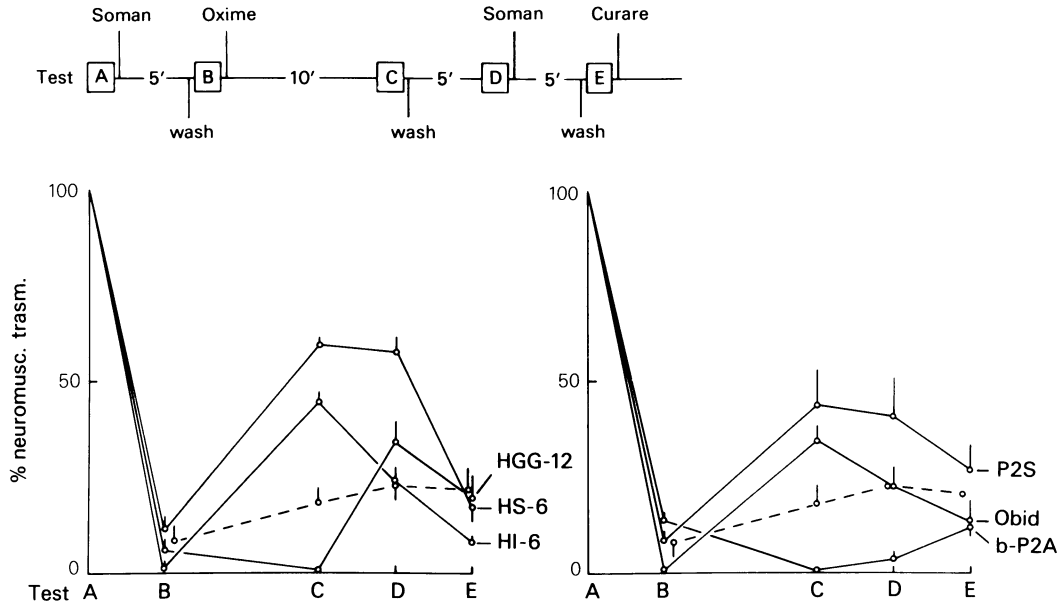

Figure 5 Neuromuscular transmission in marmoset diaphragm muscle preparations. For compounds, doses, procedures and further details see Figure 1. Each point represents the mean; s.e.means shown by vertical lines, of 4 preparations. Note that in marmoset muscles HS-6, HI-6, P2S and obidoxim are slightly effective in restoring neuromuscular transmission, whereas HGG-12 and benzyl-P2A are ineffective.

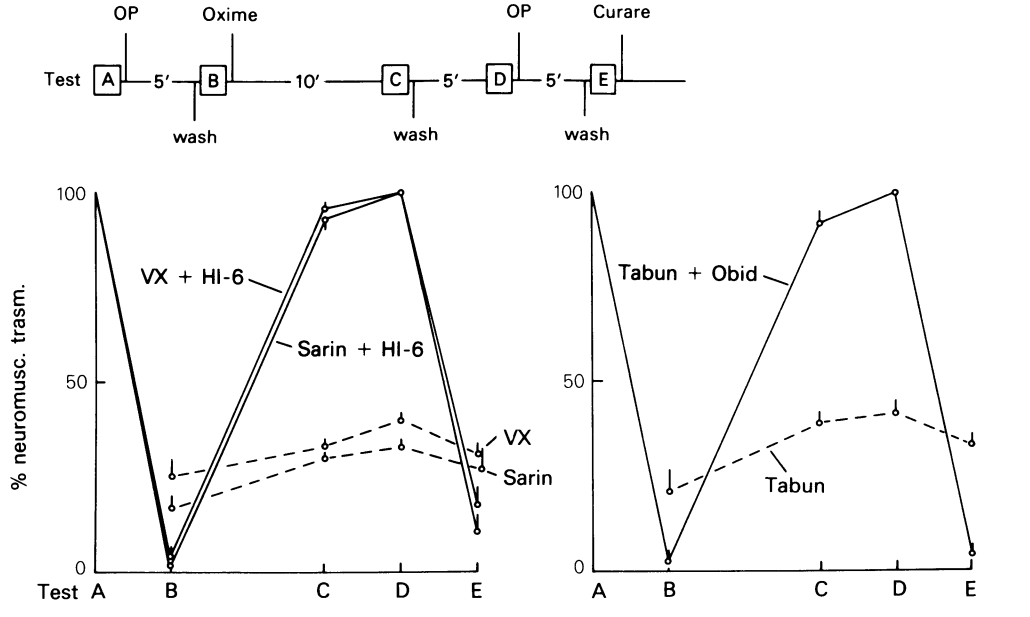

Figure 6 Neuromuscular transmission in marmoset muscle preparations. For compounds, doses and procedures see Figure 4. Each point represents the mean of 4 preparations; s.e.means shown by vertical lines; Note that in the marmoset muscle HI-6 is highly effective in restoring neuromuscular transmission after sarin or VX poisoning and that obidoxim is highly effective after tabun.

muscle in test C (Figure 2) was significantly higher than the control value. The recovery found in test C is due to the combination of direct oxime effects, enzyme reactivation and adaptation. From Figure 3 it appears that only the enzyme reactivating effect of HS-6 was significantly higher than the control value. HI-6 and obidoxim caused significant 'direct' oxime effects which were not seen with the human preparations. None of the oximes significantly affected adaptation which was in accordance with the results obtained in human preparations.

After exposure to sarin or VX, excellent recovery of the neuromuscular transmission could be obtained by treatment with HI-6 (Figure 6). Tabun poisoning

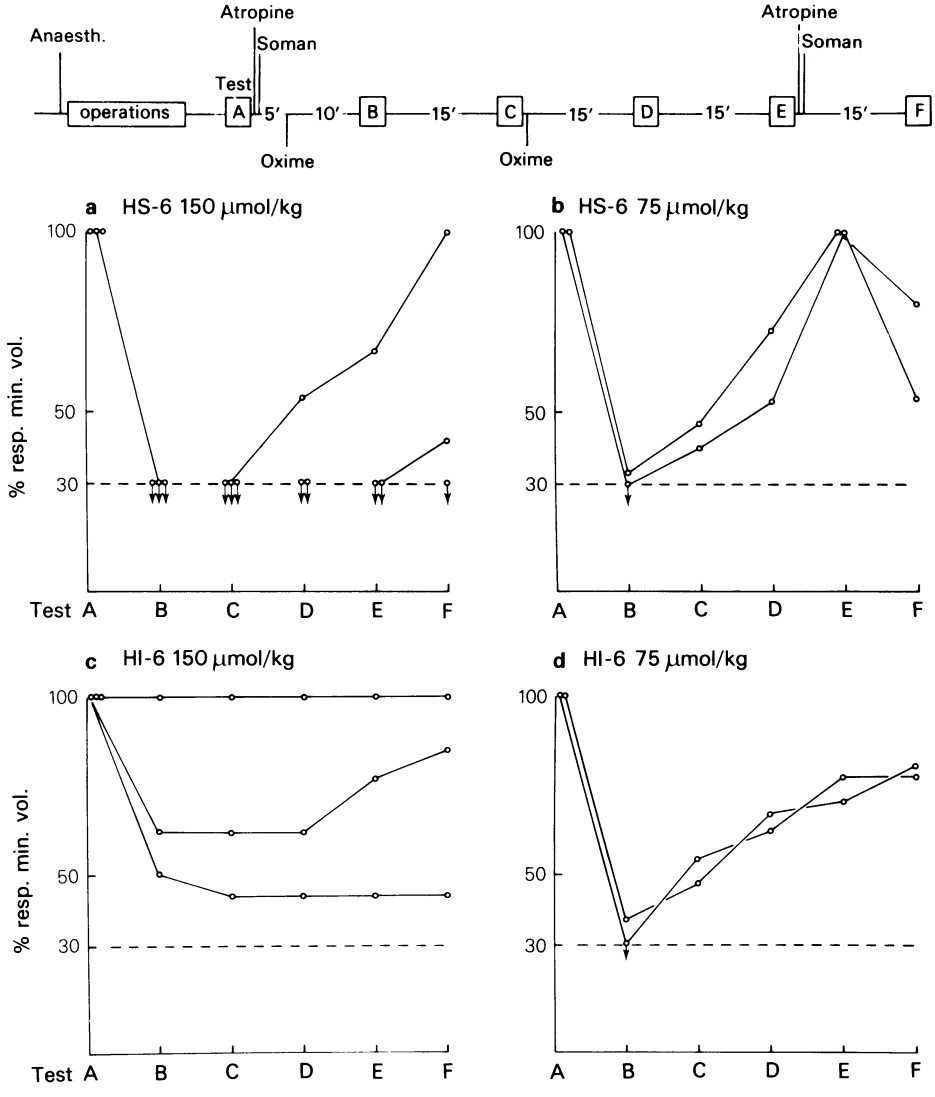

Figure 7 The individual changes in respiratory minute volume (resp. min. vol.), expressed as percentages of the control value, of anaesthetized, atropinized (10 mg/kg, i.p.), soman poisoned (32 μg/kg, i.v.) marmosets before and after oxime treatment with HS-6 or HI-6, each at i.v. doses of 150 (n= 3) or 75 μmol/kg (n= 2), according to the test procedures illustrated at the top of the figure. The second doses of the oxime (after test C), atropine and soman (after test E) were equal to the first. The interrupted lines indicate the resp. min. vol. level at which animals needed artificial respiration; the arrows denote the animals which need artificial respiration. All control animals without oxime treatment (not shown) needed artificial respiration during the total period of the experiment. The lower dose of HS-6 (b) seems to be more effective in restoring respiration than the higher dose (a). The higher dose of HI-6 (c) appears to be the most efficacious treatment.

could effectively be treated with obidoxim. Recovery observed after sarin, VX or tabun poisoning and subsequent oxim treatment, were almost completely due to the enzyme reactivating effects (D% – E%, see Figure 6) of the oximes. Control values for spontaneous recovery of neuromuscular transmission observed after soman, sarin, VX or tabun poisoning of the marmoset muscle preparation were higher than those observed in the human preparations.

In vivo *experiments*

Soman administration without oxime treatment resulted in respiratory failure within 15 min. The animals remained unable to breathe spontaneously throughout the entire 75 min of the experiment. Ten min after the administration of the higher dose of HS-6 (Figure 7a, test B), the animals needed artificial respiration. The lower dose of this oxime may be more effective; one animal did not require artificial respiration and the other one only for a brief period (Figure 7b). The second dose of soman (after test E) resulted in a partial reinhibition of the respiratory minute volume in the two animals treated with the lower dose of HS-6 showing that HS-6 had caused enzyme reactivation. HI-6 was more effective at the higher dose (compare Figure 7c with 7d) and was also more effective than HS-6 in sustaining respiration. HGG-12 was absolutely ineffective in restoring the respiratory minute volume at both dose levels.

Although all control marmosets developed respiratory paralysis, the tetanic responses of their muscles were only partially blocked (Figure 8a, test B). In contrast, in three other marmosets sarin produced a complete block of these tetanic responses at the moment of respiratory paralysis. HS-6 and HI-6, at both doses, were largely ineffective in restoring neuromuscular transmission (Figures 8a and 8b). The second dose of the oximes (after test C) caused no further changes of neuromuscular transmission. The second dose of soman after test E, administered to the oxime-treated animals, hardly reinhibited neuromuscular function. HGG-12 had a deleterious effect on neuromuscular transmission at both dose levels.

Whereas ECG patterns remained unchanged, systolic blood pressure and heart rate fell considerably after soman poisoning. These parameters were only slightly affected by HI-6 and HS-6 at the doses used. HGG-12, however, considerably increased the fall in blood pressure and heart rate.

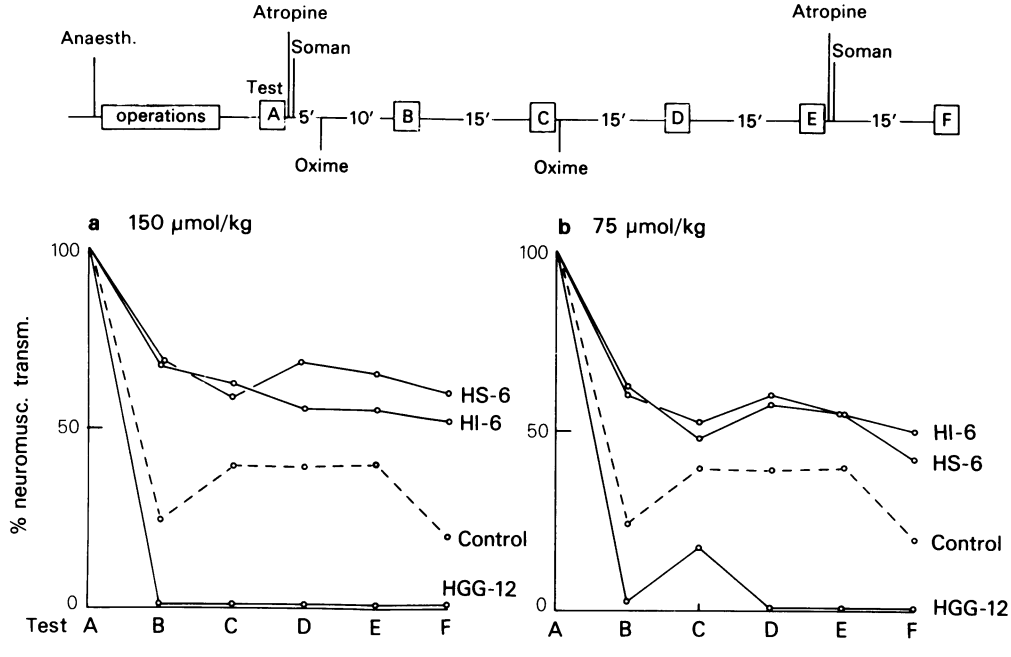

Figure 8 The percentages of neuromuscular transmission of the indirectly stimulated gastrocnemius-soleus muscles of the marmosets described in Figure 7. Although all control animals developed respiratory paralysis after soman poisoning, their neuromuscular transmission (broken line) was only partially inhibited in test B. HS-6 and HI-6, at both dose-levels were hardly effective in restoring neuromuscular transmission; a second dose of the oxime (after test C) caused no further changes of neuromuscular transmission. HGG-12 had a deleterious effect on neuromuscular transmission at both dose levels.

Discussion

Since the therapeutic efficacy of oximes in organophosphate poisoning differs considerably between species, it became necessary to find an animal that reacts as closely as possible to man. Such an animal model was particularly important since these oximes seemed to lack therapeutic effect against soman, only in the human muscle.

In the present experiments the effects of various oximes after poisoning with several organophosphates were tested on the marmoset muscle preparation and on the intact marmoset. The effects were compared with those in the human muscle preparation. In the comparisons the following effects were noteworthy: (1) It was obvious that in general the oxime-efficacy in the soman-poisoned human muscle preparation was low. The effects found in the marmoset preparation resembled those found in the human preparation in that the oxime-efficacy after soman-poisoning was much lower than previously found in rodents and dogs (Wolthuis *et al.*, 1981). A possible reason for the much lower efficacy of oximes against soman poisoning of muscle preparations from marmosets and man could be a higher rate of 'ageing' of the soman-AChE in these species. Alternatively, it might be that there exist subtle species differences in the AChE-molecule, which renders reactivation by oximes more difficult. There were, however, subtle differences between the marmoset and the human muscle with respect to the comparative efficacy of the individual oximes, in particular with respect to P2S. (2) HI-6 and obidoxim exerted a 'direct' effect in marmoset muscles but not in human muscles. (3) The poor recovery of neuromuscular transmission after oxime treatment was restricted to soman-poisoned muscles. After sarin, VX or tabun-poisoning excellent recovery could be obtained with suitable oximes in muscles from both species. In addition, these experiments demonstrated that the poor recovery after soman-poisoning could not be attributed to the methods applied. (4) The therapeutic effects of HGG-12 in the soman-poisoned muscles from both species were negligible, often the effects were even adverse, which is in marked contrast with findings in dogs (Hauser & Weger, 1979; Weger & Szinicz, 1981) or dog muscles (Wolthuis *et al.*, 1981). Because HGG-12 has ganglionic blocking effects (Kirsch & Weger, 1981) and affects the blood pressure in dogs (Hauser & Weger, 1979) as well as in marmosets (present results), lower doses were also tested in the muscles of man and marmoset as well as in the intact marmoset. In the intact animal 50% of the standard dose of 150 μmol/kg i.v. did not result in an improvement; *in vitro* the stepwise lowering of the HGG-12 dose to 0.375 mM (marmoset muscle) or to 0.05 mM (human muscle) did not cause a better result than in control preparations without oxime.

In a few separate experiments it was established that recovery in the diaphragm, the intercostal muscles and the gastrocnemius-soleus muscles of the marmoset followed essentially the same pattern. The degree of the neuromuscular transmission in these muscles, therefore, was considered to be equally important for the determination of inhibitory or recovery processes.

As was previously found in the rat, it was now established that in the marmoset, sarin has its effects predominantly in the periphery and soman predominantly in the central nervous system (CNS). It was therefore necessary to measure the oxime efficacy also *in vivo* to determine whether the oximes had therapeutic effects against the central action of soman. HS-6 and HI-6 penetrate into the CNS (Clement, 1981; Wolthuis *et al.*, 1981). Because HS-6 in the soman-poisoned marmoset muscle was more effective *in vitro* than HI-6, it was surprising that *in vivo* HI-6 appeared to be the most effective of the two. Whether this has to be explained in terms of central oxime efficacy or in terms of differences in the pharmacokinetics of these two oximes was not further investigated.

In spite of the administration of 10 mg/kg of atropine sulphate to the animals, cardiovascular effects were observed after soman administration, i.e. a considerable drop in blood pressure as well as in heart rate. The use of 4 mg/kg of atropine sulphate in our experiments resulted in a lethal decrease of blood pressure after soman poisoning and was obviously too low. Since the blood pressure lowering effect and the bradycardia appeared not to be abolished by 10 mg/kg atropine sulphate and in a few separate experiments not even by 20 mg/kg, these cardiovascular effects of soman appear to be insensitive to atropine. This may be an effect in the CNS. Support for such a central action of soman on heart rate and blood pressure came from the finding in comparable experiments with the predominantly peripheral acting organophosphate, sarin ($4 \times LD_{50}$ i.v.), in which the same doses of atropine caused hardly any cardiovascular effects. With a cross-perfusion technique in rabbits, Preston & Heath (1972) found similar effects on blood pressure and heart rate after soman-intoxication which were also insensitive to atropine treatment.

The main goal of the present study was to investigate whether oxime-effects in the organophosphate-poisoned marmoset muscle bear a greater similarity to those in the human muscle than the previously observed effects in muscles from rodents and dogs. In spite of some differences the marmoset muscle appears to be a reasonable model for the human muscle to test the efficacy of oximes.

The staffs of the Thoracic Surgery Centres of the Erasmus University in Rotterdam (head: Prof. J. Nauta) and of the Leiden University (head: Prof. A.G. Brom) are hereby gratefully acknowledged for their cooperative spirit. Many thanks are due to Mr R.A.P. Vanwersch and Mr A.W. Kluivers of the Medical Biological Laboratory TNO for their technical assistance and to Dr M. Wijnans for statistical evaluation of the results.

References

ADAMS, G.K., YAMAMURA, H.I. & O'LEARY, F.F. (1976). Recovery of central respiratory function following anticholinesterase intoxication. *Eur. J. Pharmac.*, **38,** 101–112.

BARTLETT, M.S. (1937). Properties of sufficiency and statistical tests. *Proc. R. Soc.* A160, 268.

BENSCHOP, H.P., KONINGS, K.A.G., KOSSEN, S.P. & LIGTENSTEIN: D.A. (1981). Determination of some pyridinium aldoxime compounds by means of ion-pair reversed-phase high-performance liquid chromatography: application in biological material. *J. Chromatogr.*, **225,** 107–114.

CLEMENT, J.G. (1981). Toxicology and pharmacology of bispyridinium oximes. Insight into the mechanism of action vs soman poisoning *in vivo*. *Fundam. appl. Toxic.*, **1,** 193–202.

DIRNHUBER, P., FRENCH, M.C., GREEN, D.M., LEADBEATER, L. & STRATTON, J.A. (1979). The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J. Pharm. Pharmac.*, **31,** 295–299.

HAUSER, W. & WEGER, N. (1979). Therapeutic effects of the bis-pyridinium salts HGG-12, HGG-42 and atropine, benactyzine in organophosphate poisoning in dogs. *Arch. Toxicol. Suppl.*, **2,** 393–396.

KEPNER, L.A. & WOLTHUIS, O.L. (1978). A comparison of the oximes HS-6 and HI-6 in the therapy of soman intoxication in rodents. *Eur. J. Pharmac.*, **48,** 377–382.

KIRSCH, D.M. & WEGER, N. (1981). Effects of the bispyridinium compounds HGG-12, HGG-42, and obidoxim on synaptic transmission and NAD (P) H-fluorescence in the superior cervical ganglion of the rat in vitro. *Arch. Toxicol.*, **47,** 217–232.

MEETER, E. (1969). Desensitization of the end-plate membrane following cholinesterase inhibition, an adjustment to a new working situation. *Acta physiol. pharmac. néerl.*, **15,** 243–258.

MEETER, E. & WOLTHUIS, O.L. (1968). The spontaneous recovery of respiration and neuromuscular transmission in the rat after anticholinesterase poisoning. *Eur. J. Pharmac.*, **2,** 377–386.

MEETER, E., WOLTHUIS, O.L. & BENTHEM, R.M.J. VAN (1971). The anticholinesterase hypothermia in the rat: its practical application in the study of the central effectiveness of oximes. *Bull. Wld. Hlth. Org.*, **44,** 251–261.

MILLER, R.G. (ed) (1966). *Simultaneous Statistical Inference.* pp. 67–70 and pp. 81–89. New York: McGraw-Hill.

PRESTON, E. & HEATH, C. (1972). Depression of the vasomotor system in rabbits poisoned with an organophosphate anticholinesterase. *Archs int. Pharmacodyn.*, **200,** 245–254.

SMITH, A.P., WIEL, H.J. VAN DER & WOLTHUIS, O.L. (1981). Analysis of oxime-induced neuromuscular recovery in guinea-pig, rat and man following soman poisoning *in vitro*. *Eur. J. Pharmac.*, **70,** 371–379.

SMITH, A.P. & WOLTHUIS, O.L. (1983) HI-6 as an antidote to soman poisoning in rhesus monkey respiratory muscles *in vitro*. *J. Pharm. Pharmac.*, (in press).

WEGER, N. & SZINICZ, L. (1981). Therapeutic effects of new oximes, benactyzine and atropine in soman poisoning: Part I. Effects of various oximes in soman, sarin, and VX poisoning in dogs. *Fundam. appl. Toxic.*, **1,** 161–163.

WOLTHUIS, O.L. & KEPNER, L.A. (1978). Successful oxime therapy one hour after soman intoxication in the rat. *Eur. J. Pharmac.*, **49,** 415–425.

WOLTHUIS, O.L., BERENDS, F. & MEETER, E. (1981). Problems in the therapy of soman poisoning. *Fundam. appl. Toxic.*, **1,** 183–192.

WOLTHUIS, O.L., VANWERSCH, R.A.P. & WIEL, H.J. VAN DER (1981). The efficacy of some bis-pyridinium oximes as antidotes to soman in isolated muscles of several species including man. *Eur. J. Pharmac.*, **70,** 355–369.

(Received September 10, 1982. Revised October 28, 1982.)