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MINIMUM CONCENTRATIONS OF N-METHYLPYRIDINIUM-2-ALDOXIME METHANE SULPHONATE (P2S) WHICH REVERSE NEUROMUSCULAR BLOCK

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Abstract—The effects of different concentrations of N-methylpyridinium-2-aldoxime methane sulphonate (P2S) on the neuromuscular block produced by methyl*iso*propoxy-phosphoryl fluoride (sarin), methyl*iso*propoxy-(2-dimethylaminoethylthio)-phosphine oxide (37 S-N) and methyl*iso*propoxyphosphoryl thiocholine (37 S-N$^+$) have been studied.

The block produced by the three inhibitors on the rat phrenic nerve diaphragm preparation is reversed by 1×10^{-5} M P2S. The time required to produce 50 per cent reversal of the block was the same against all inhibitors.

In anaesthetized cats poisoned with lethal doses of 37 S-N$^+$ plasma concentrations of about 2×10^{-5} M (4 μg/ml) were needed to counteract neuromuscular block, bradycardia, hypotension and respiratory failure.

ONE of the most effective antidote combinations against nerve gas poisoning is N-methylpyridinium-2-aldoxime with atropine. The oxime effectively ameliorates symptoms such as muscle weakness and paralysis thus decreasing the need for artificial respiration.[1] Plasma concentrations after intramuscular and oral administration of the oxime have previously been determined in man[2] but the concentrations needed for therapeutic effect, have not been established.

The aim of the present investigation was to determine the minimum concentrations of N-methylpyridinium-2-aldoxime methane-sulphonate (P2S) which reverse neuromuscular block caused by organophosphorous cholinesterase inhibitors.

MATERIALS*

P2S, N-methylpyridinium-2-aldoxime methane sulphonate was synthetized according to the method of Creasy and Green.[3] 37 S-N, methyl*iso*propoxy-(2-dimethylamino-ethylthio)-phosphine oxide acid oxalate and 37 S-N$^+$, methyl*iso*propoxyphosphory thiocholine iodide were synthetized according to the method of Tammelin,[4] sarin, methyl*iso*propoxyphosphoryl fluoride according to the method of Boquet,[5] and tabun, dimethylamidoethoxy-phosphoryl cyanide according to Holmstedt.[6]

METHODS

Rat phrenic nerve diaphragm preparation

The method was that described by Fredriksson and Tibbling.[7] If not otherwise stated the preparation was washed twice with Tyrode's solution before addition of

* The compounds were synthetized by Miss Inger Enander and Mr. Lars Fagerlind.

oxime in order to remove excess inhibitor. Control experiments showed that there was negligible reversal of twitch height after this washing procedure. The concentration of inhibitor in the bath was 5×10^{-7} M, which produced almost complete block within 30 min. As seen in Table 1 the time required for the development of maximum neuromuscular block, varies with the organophosphate. The preparation was consequently washed after 12 min in the case of sarin and after 30 min in the case of 37 S-N or 37 S-N^+ in order to obtain block of approximately the same magnitude. After washing P2S was added to a final concentration of from 5×10^{-6} to 1×10^{-3} M and the time required for the contractions to return to 50 per cent of their original value was then determined. It was found that the washing procedure did not significantly change the time required for 50 per cent reversal (in these experiments 5×10^{-4} M P2S was used).

TABLE 1. TIME REQUIRED FOR NEUROMUSCULAR BLOCKING EFFECT OF SARIN, 37 S-N, 37 S-N^+ AND TABUN 5×10^{-7} M ON THE ISOLATED RAT PHRENIC NERVE DIAPHRAGM PREPARATION

(The nerve was stimulated at 30 and 50 cycles/sec for 3 sec every 30 sec.)

Inhibitor	Time to maximum block in minutes (mean ± s.e.) Stimulation frequency	
	30 c/s	50 c/s
Sarin	6·7 ± 0·7 (n = 7)	7·3 ± 0·7 (n = 7)
37 S-N	21·6 ± 0·8 (n = 10)	22·8 ± 0·8 (n = 9)
37 S-N^+	16·5 ± 0·6 (n = 9)	17·1 ± 0·5 (n = 8)
Tabun	9·6 ± 0·7 (n = 5)	10·2 ± 0·8 (n = 5)

Frog rectus abdominis muscle

Receptor effects of the quaternary inhibitor 37 S-N^+ were tested according to Fredriksson and Tibbling.[8] The rectus muscle was incubated with sarin (5×10^{-5} M for 30 min) and then washed repeatedly. This concentration of sarin produces a complete inactivation of the cholinesterase of the muscle. 37 S-N^+ was then tested in increasing concentrations. Acetylcholine was used as reference.

Determination of therapeutic plasma concentrations

The experiments were performed on cats anaesthetized with sodium pentobarbital (30–40 mg/kg) intraperitoneally. Blood pressure, heart rate, respiration and contractions of the indirectly stimulated gastrocnemius–soleus muscles were recorded in all experiments. The recording technique was that described by Lindgren and Sundwall.[9]

A lethal dose of inhibitor was administered intravenously followed by intramuscular injections of P2S (in the biceps femoris of the leg opposite to that being stimulated) at different time intervals. Blood samples were taken from the femoral artery of the injected side before injection of organophosphate, at signs of improvement, after complete reversal of recorded symptoms and at the end of the experiment which usually lasted for from 2 to 3 hr. The samples (about 5 ml each) were collected in heparinized centrifuge tubes and the plasma analyzed for P2S.[2] Five millilitres of heparinized cat blood and 2 ml of Macrodex (R) were injected intra-arterially after each blood sample.

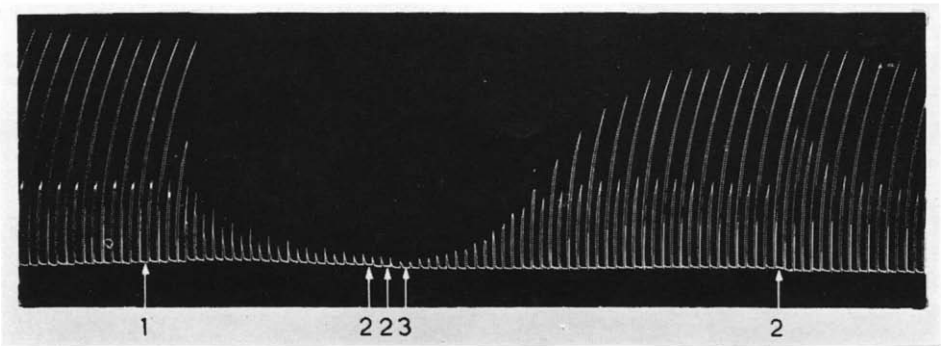

FIG. 2. Effect of P2S (5×10^{-5} M) on the neuromuscular block produced by sarin (5×10^{-7} M). Rat phrenic nerve diaphragm preparation. The nerve was stimulated with 30 and 50 c/s for 3 sec twice a minute. At (1) addition of sarin, at (2) washing and at (3) addition of the oxime.

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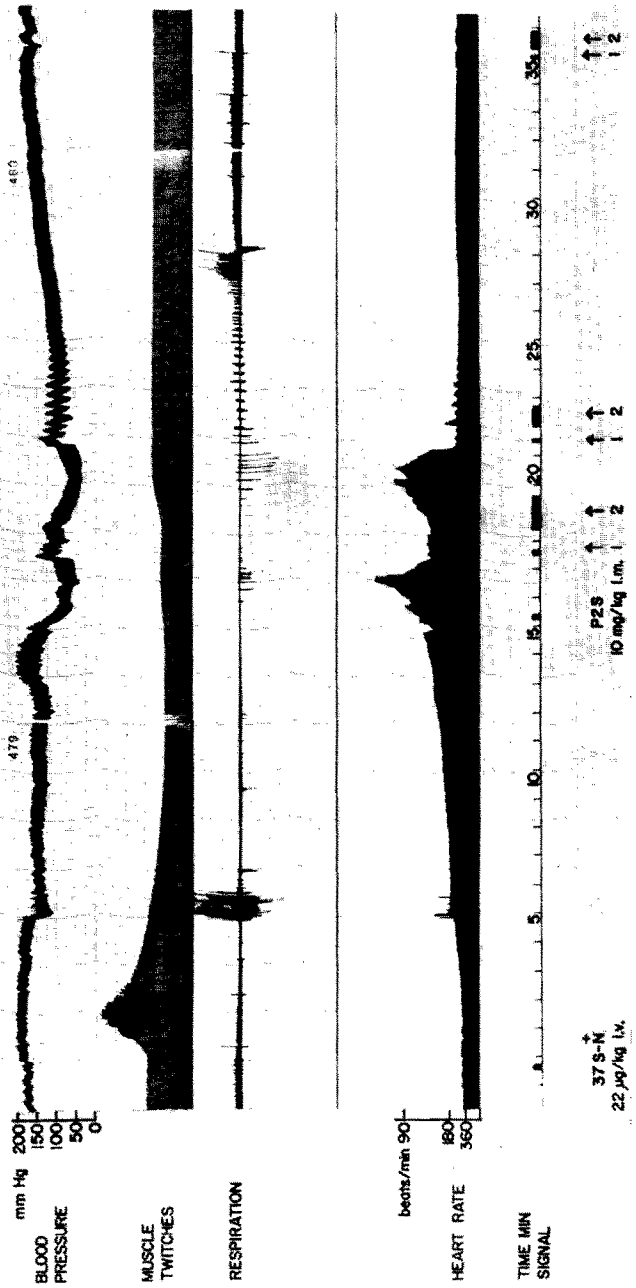

FIG. 3. Effect of P2S (10 mg/kg intramuscularly) on blood pressure, neuromuscular transmission, respiration and heart rate in anaesthetized cat poisoned with 37 S-N[1] (22 μg/kg intravenously). At (1) collection of blood samples, at (2) injection of blood and macrodex. The concentrations of P2S in the samples were 8, 9 and 12 μg/ml plasma, respectively. The sciatic nerve was continuously stimulated with 0·3 c/s. Time in minutes.

RESULTS

Effect of P2S on neuromuscular block produced by sarin, 37 S-N, 37 S-N$^+$ and tabun on rat phrenic nerve diaphragm preparation

The results are summarized in Fig. 1 and a typical experiment is shown in Fig. 2. It is seen that the 50 per cent reversal time was the same against sarin, 37 S-N and 37 S-N$^+$ at oxime concentrations of 5×10^{-5} M to 5×10^{-4} M. At a concentration of 5×10^{-6} M, P2S failed to achieve 50 per cent reversal within 30 min in seven out of eighteen experiments and in the experiments where 50 per cent reversal was achieved, the time for this effect varied. It is also seen that the block produced by tabun is reversed much more slowly.

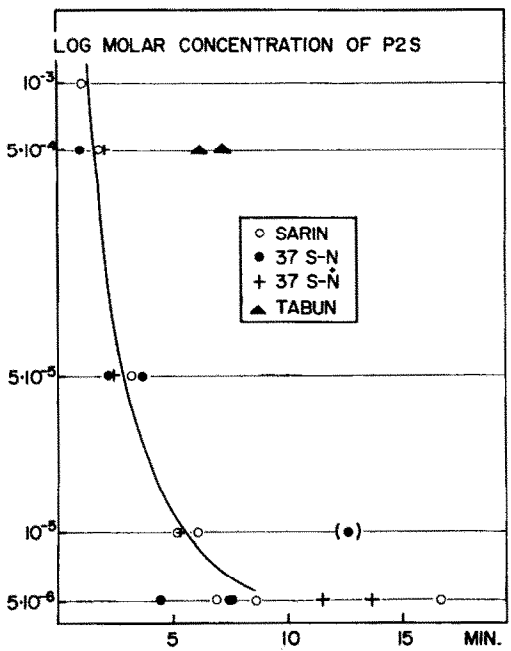

FIG. 1. Effect of P2S on time required for 50 per cent reversal of the neuromuscular block produced by sarin, 37 S-N, 37 S-N$^+$ and tabun (5×10^{-7} M) (the curve is drawn for sarin). Rat phrenic nerve diaphragm preparation. The nerve was stimulated with 30 and 50 cycles/sec for 3 sec twice a minute.

Effects of 37 S-N$^+$ on cholinergic receptors

The inhibitor evoked contractions of the sarin treated frog rectus abdominis muscle, but only at concentrations higher than 10^{-3} M. This concentration is much higher than might be expected in blood of animals poisoned by lethal doses. It is therefore less likely that receptor effects contribute to the toxicity of the drug.

Therapeutic plasma concentration of P2S in the anaesthetized cat

In preliminary experiments it was found that 22 μg/kg of 37 S-N$^+$, given intravenously, is a lethal dose, death occurring within 18–25 min.

In three experiments 10 mg/kg of P2S was injected intramuscularly at the appearance of symptoms after intravenous injection of 22 μg/kg of 37 S-N$^+$. Most toxic symptoms were reversed within 30 min and the plasma concentration of P2S was

from 4 to 12 μg/ml, when blood pressure, heart rate and respiration was normal. A typical experiment is shown in Fig. 3. P2S counteracts the severe bradycardia, re-establishes a normal blood pressure and spontaneous breathing. The increase in twitch height of the gastrocnemius muscle is simultaneous with the return of spontaneous respiratory activity. The plasma concentrations of P2S were about 8 μg/ml in the blood samples collected at the beginning of improvement and from 9 to 12 μg/ml when blood pressure, bradycardia and respiration became normal.

In two experiments the dose of 37 S-N^+ was increased to 27 μg/kg and P2S (10 mg/kg) was injected intramuscularly. The results were essentially the same as those obtained with the lower dose of inhibitor. The plasma concentration of P2S was about 8 μg/ml when blood pressure, heart rate and respiration became normal.

The dose of P2S was then decreased to 5 mg/kg in five experiments. In most cases the recorded symptoms were not fully reversed or were reversed only for a short time. The plasma concentrations were then from 2 to 4 μg/ml. A typical experiment is illustrated in Fig. 4. Four animals survived for more than 2 hr, one died after 30 min. No P2S was found in the plasma of the animal which died.

In the two first series of experiments above, neuromuscular block was generally not detected. In most cases only a potentiation of twitch height was seen before the injection of P2S. In the third series tetanic stimulation was performed instead of single twitches in order to obtain records on the effect of P2S on neuromuscular block. In these experiments the ability of the gastrocnemius to sustain a tetanus was decreased by from 20 to 95 per cent before injection of P2S. Improvement after P2S was always seen, but complete reversal only in one case.

In the experiments described bronchoconstriction and salivation were not measured, but it was noted that the dyspnoea and the increased flow of saliva were not relieved by P2S.

Experiments were also undertaken to demonstrate that the therapeutic effect of P2S could not entirely be due to an atropine-like effect (Fig. 5). Atropine prevents the bradycardia, but not the respiratory failure, in contrast to P2S which promptly restores normal respiration.

DISCUSSION

The results obtained indicate that plasma levels of P2S above 4 μg/ml counteract neuromuscular block *in vitro* and *in vivo*, and bradycardia, hypotension and respiratory failure *in vivo*. Atropine was not used in the experiments and it is therefore possible that the concentrations of P2S together with atropine needed for therapeutic effect might be lower, since atropine may prolong survival, thus giving the oxime more time to reactivate the inhibited enzyme.

If the results are valid in man, intramuscular injection of from 20 to 30 mg P2S per kg body weight should yield therapeutic plasma concentrations after from 5 to 10 min.[2] Effective plasma concentrations are also reached after oral administration, but as the rate of absorption is slow, the oxime must then probably be administered before poisoning.

P2S is equally effective against the neuromuscular block produced by sarin, 37 S-N and 37 S-N^+ (which yield the same type of phosphorylated enzyme) but is much less effective against the block produced by tabun. As it has been shown that methyl*iso*-propoxy phosphorylated cholinesterase is reactivated much more readily than that

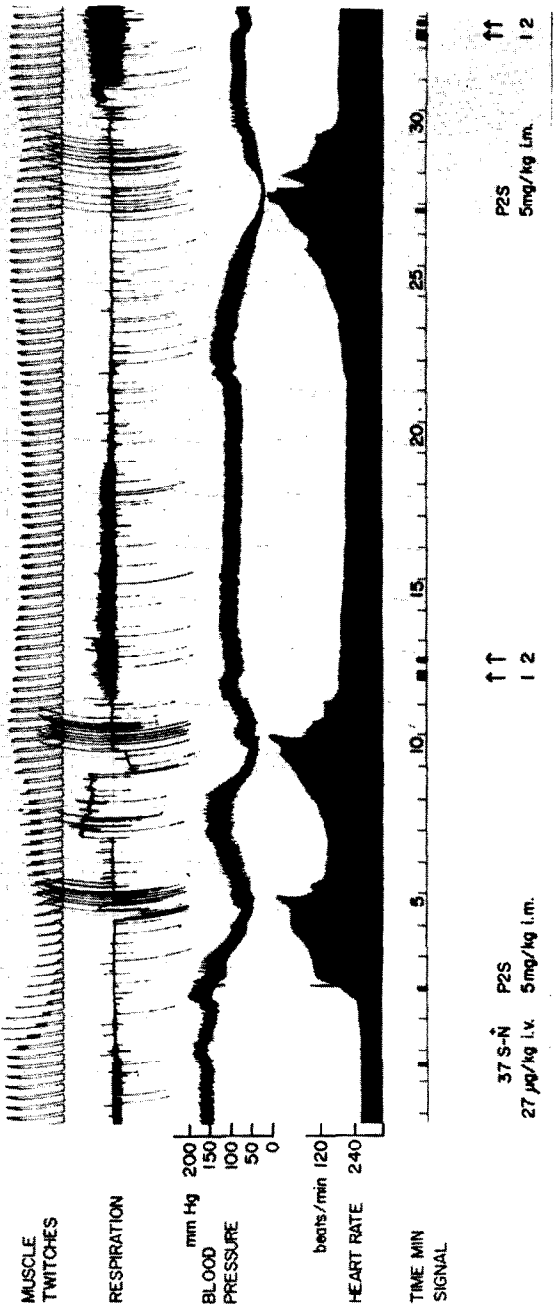

FIG. 4. Effect of P2S (5 mg/kg intramuscularly) on neuromuscular transmission, respiration, blood pressure and heart rate in anaesthetized cat poisoned with 37 S-N (27 μg/kg intravenously). At (1) collection of blood samples at (2) injection of blood and macrodex. Between the samples another intramuscular injection of 5 mg/kg of P2S was given. The concentrations of P2S was 2 and 3 μg/ml plasma, respectively. The sciatic nerve was stimulated with 20 c/s for 5 sec every 20 sec. Time in minutes.

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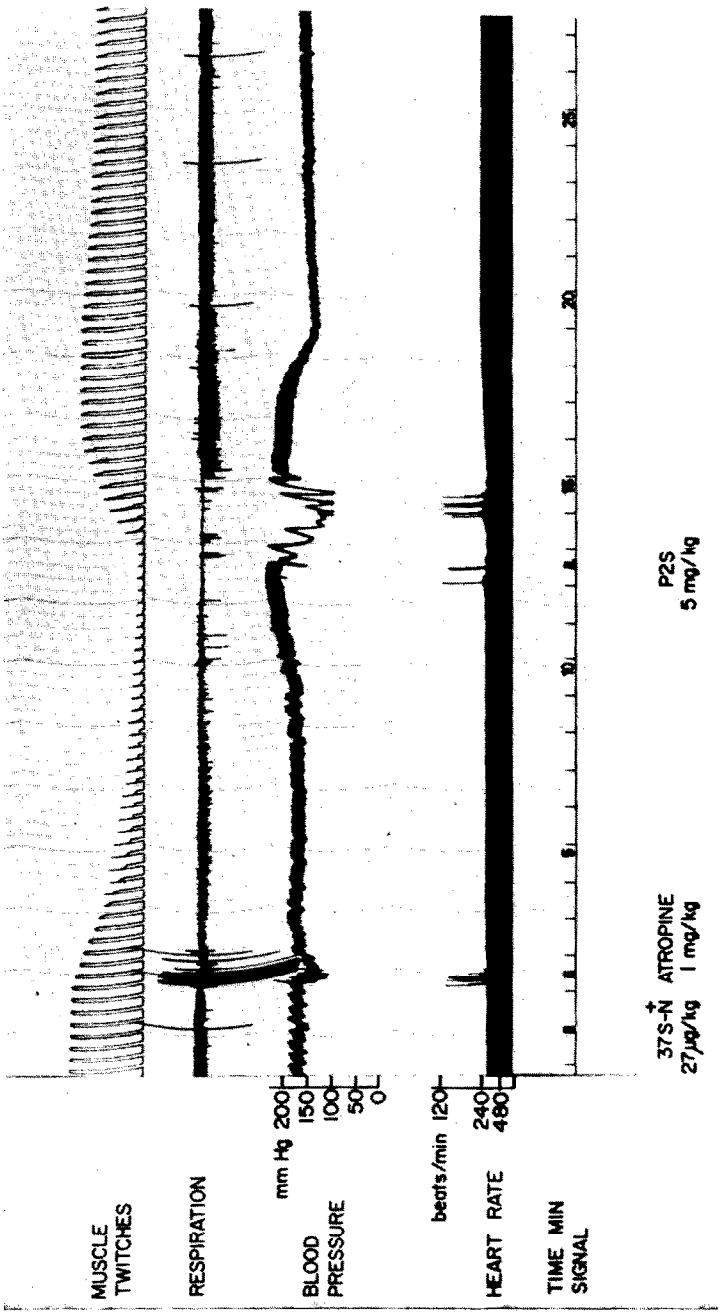

FIG. 5. Effect of P2S (5 mg/kg intravenously) on neuromuscular transmission, respiration, blood pressure and heart rate in anaesthetized cat poisoned with 37 S-N[1] (27 μg/kg intravenously) and treated with atropine (1 mg/kg intravenously). The sciatic nerve was stimulated with 20 cycle/sec for 5 sec every 20 sec. Time in minutes.

inhibited by tabun[11] the experiments described support the concept that the oxime acts at the neuromuscular junction principally by reactivation of the phosphorylated cholinesterase.

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REFERENCES

1. B. HOLMSTEDT, *Pharmacol. Rev.* **11**, 567 (1959).
2. A. SUNDWALL, *Biochem. Pharmacol.* **5**, 225 (1960).
3. N. H. CREASY and A. L. GREEN, *J. Pharm., Lond.* **11**, 485 (1959).
4. L.-E. TAMMELIN, *Acta Chem. Scand.* **11**, 1340 (1957).
5. J. R. BOQUET, *Acta Med Belg., Bruxelles* (1956).
6. B. HOLMSTEDT, *Acta Physiol. Scand.* **25**, suppl. (1951).
7. T. FREDRIKSSON and G. TIBBLING, *Biochem. Pharmacol.* **2**, 63 (1959).
8. T. FREDRIKSSON and G. TIBBLING, *Biochem. Pharmacol.* **2**, 286 (1959).
9. P. LINDGREN and A. SUNDWALL, *Acta Pharm. Tox. Kbh.* **17**, 69 (1960).
10. L.-E. TAMMELIN, *Ark. kem.* **12**, 287 (1958).
11. I. ENANDER. Personal communication.