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ANTIDOTE EFFECT OF SODIUM FLUORIDE IN ORGANOPHOSPHORUS ANTICHOLINESTERASE POISONING

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Abstract—The effect of sodium fluoride in Sarin, Tabun and $^{37}S—N^+$ (the thiocholine analogue of Sarin) poisoning was studied. In mice sodium fluoride alone could not protect against Sarin, but had a substantial antidote effect against $^{37}S—N^+$. When given prophylactically together with atropine, NaF (20 mg/kg) increased the LD_{50} of Sarin 4·5 times, that of $^{37}S—N^+$ 7·5 times and that of Tabun 2·5 times. Therapeutical effect in Sarin and Tabun poisoning was noticed. Brain and particularly muscle ChE from Sarin poisoned mice was found to be higher after previous administration of NaF and the ChE activity of the erythrocytes from Sarin poisoned dogs increased upon administration of NaF. In anaesthetized Sarin poisoned cats NaF restores the blocked neuromuscular function. The effect on the bradycardia and on the blood pressure is small.

RECENTLY cholinesterase (ChE) reactivating properties of sodium fluoride have been demonstrated *in vitro*.[1, 2] In this paper the effect of sodium fluoride in animals poisoned with some organo-phosphorus compounds is studied.

MATERIALS AND METHODS

Estimation of therapeutic efficacy

The experiments were performed with CBA mice. Methyl-isopropoxy-phosphoryl fluoride (Sarin), methyl-isopropoxy-phosphoryl thiocholine iodide (^{37}S-N^+) and dimethylamido-ethoxy-phosphoryl cyanide (Tabun) were given subcutaneously; atropine, sodium fluoride (NaF) and N-methylpyridinium-2-aldoxime methane sulphonate (P2S) intraperitoneally. Injection volumes of the drugs were 10 ml/kg body wt. When antidote combinations were tested, the drugs were dissolved together. The observation time was 24 hr. The therapeutic efficacy is expressed as the increase in LD_{50} (multiples) produced by the treatment. LD_{50} was calculated according to Miller and Tainter.[3] For Tabun and ^{37}S-N^+ only three points in the probit range 3–7 on the regression line were determined.

Determination of cholinesterase activity in brain and muscle of mice

Three groups of five mice each were pretreated with intraperitoneal injections of either atropine (10 mg/kg), atropine (10 mg/kg) and NaF (20 mg/kg) or atropine (10 mg/kg) and P2S (30 mg/kg). Ten minutes later one LD_{50} of Sarin was given subcutaneously. The animals were killed after 2 hr by decapitation. Samples of brain and skeletal muscle were taken immediately for ChE determination.

Experiments described in the literature[4] have indicated that tissues of animals poisoned with organophosphorus compounds may contain 'free' inhibitor, which

during homogenization continues to inhibit enzyme located inside the cells. To avoid this some authors have freeze dried and/or extracted tissues prior to homogenization.[4,5] However, under the conditions used in our experiments (see below) no difference in ChE activity was found between freeze dried and only homogenized brain and muscle. Freeze drying was therefore omitted. The tissues were homogenized with an ice-chilled Potter–Elvehjem homogenizer using 0·2 g brain (0·6 g muscle) per ml distilled water. The homogenates were further diluted 1 → 10 (1 → 5) with cold distilled water. For determination of enzyme activity 3 ml of the diluted homogenate were mixed with 1·5 ml of Michels buffer and 1·5 ml of distilled water. ChE activity was measured electrometrically[6] at pH 8 and 25° with a final acetylcholine iodide concentration of $7{\cdot}3 \times 10^{-3}$M.

Determination of cholinesterase activity in erythrocytes of dogs

Three unanaesthetized dogs of 10–20 kg weight were intoxicated with Sarin by intravenous injection of 10–25 μg/kg. Ninety min later 20 mg/kg NaF were given intravenously. Another dose of 10 mg/kg NaF was given 2·5–3·5 hr later. A fourth dog obtained only one dose of 20 mg/kg NaF intravenously 4 hr after the Sarin intoxication. All Sarin poisoned dogs showed symptoms of anticholinesterase poisoning such as salivation, fasciculations and tremor. In a fifth dog, not intoxicated with Sarin, the effect of NaF *per se* on blood ChE activity was studied. 20 mg/kg NaF were given intravenously, followed by 10 mg/kg NaF 2 hr later. In this dog vomiting occurred. Venous blood samples were collected in chilled heparinized test tubes prior to the administration of Sarin (NaF) and at different time intervals after the injection of Sarin (NaF). The erythrocytes were washed twice with 10 volumes of ice-cold saline and then diluted with one volume of distilled water. The ChE activity was determined as described above.

Recording of respiration, blood pressure, heart rate and muscular contractions

The effects of sodium fluoride were studied in ten Sarin poisoned cats anaesthetized with intraperitoneal injections of sodium pentobarbital (30–40 mg/kg); supplementary doses were given intravenously during the course of the experiment. Drugs were injected intravenously through a plastic catheter into a femoral vein or intra-arterially through a cannula in the femoral artery of the leg opposite to that being stimulated (see below). In the latter case the tip of the cannula was lying proximally to the aortic bifurcation. The parameters recorded were obtained from the output of suitably arranged transducers to a Grass Model 5 Polygraph. *Respiration* was recorded as pressure differences in the tracheal cannula by means of a pressure transducer (Model PT 5, Grass Instrument Corporation). *Blood pressure* was recorded from the left carotid artery by means of a Statham electromanometer. *Heart rate* was estimated by the use of a modification of an interval recorder described by Goldschmidt and Lindgren.[7] The effects of the drugs on *neuromuscular transmission* were studied by recording isometric contractions of the gastrocnemius muscle in response to electrical stimulation of the sciatic nerve. (Grass stimulator, Model S4). The nerve was stimulated with 20 c/s for 5 sec every 20 sec (duration 5 msec, 0·4–0·6 V). Shielded silver electrodes were applied to the distal part of the sectioned nerve. A force displacement transducer Model PT 10 (Grass Instrument Corporation) was used for recording the muscle contractions.

RESULTS

Antidote effect of NaF in Sarin, ^{37}S-N^+ and Tabun poisoned mice

The intraperitoneal LD_{50} of NaF was found to be 34 ± 1·5 mg/kg. As seen in Table 1 NaF alone (10 and 20 mg/kg) gives practically no protection against Sarin. A combination of NaF (20 mg/kg) and atropine (10 mg/kg) on the other hand increases the LD_{50} of Sarin 4·5 times. With a lower dose of NaF (10 mg/kg) the effect was less pronounced. A combination of atropine and P2S (10 and 30 mg/kg) under the same experimental conditions increases the LD_{50} of Sarin 6·4 times. No further increase in protection is afforded with the addition of NaF. Given 30 sec after poisoning NaF + atropine increase the LD_{50} of Sarin with a factor of 2·6.

Given prophylactically NaF alone (20 mg/kg) was found to have substantial antidote effect against ^{37}S-N^+, a drug which penetrates poorly into the central nervous system.[8] The LD_{50} increased 2·5 times and in the presence of atropine (10 mg/kg) the efficacy increased further to 7·5 LD_{50} (Table 1).

TABLE 1. INCREASE IN LD_{50} (MULTIPLES) OF SARIN AFTER DIFFERENT ANTIDOTES GIVEN EITHER PROPHYLACTICALLY OR THERAPEUTICALLY TO MICE

Antidote			Multiples of LD_{50}	
Atropine (mg/kg i.p.)	NaF (mg/kg i.p.)	P2S (mg/kg i.p.)	Treatment 10 min before poisoning	Treatment ½ min after poisoning
			Sarin s.c.	
10	—	—	~1	—
10	10	—	2·8	—
10	20	—	4·5	2·6
—	10	—	~1	—
—	20	—	~1	—
10	—	30	6·4	—
10	20	30	6·4	—
			^{37}S—N^+ s.c.	
—	20	—	~2·5	—
10	20	—	~7·5	—
			Tabun s.c.	
10	—	—	~1·3	~1
10	20	—	~2·5	~2·4

In Tabun poisoning NaF in combination with atropine (10 mg/kg) increases the LD_{50} by a factor of 2·5, either the drugs were given prophylactically or therapeutically (Table 1).

Table 2 shows that the protective effect of NaF in combination with atropine (10mg/kg) markedly decreases with increase in the time interval between injections of NaF (20mg/kg) and Sarin (4 LD_{50}).

Cholinesterase activities in brain and muscle in Sarin poisoned and NaF treated mice

Following one LD_{50} of Sarin the ChE activity of muscle and brain from animals treated only with atropine is 56 and 20 per cent respectively of the normal value 2 hr after intoxication. When the animals were pretreated with a combination of atropine and sodium fluoride higher values of enzyme activity, 88 and 33 per cent respectively,

TABLE 2. DECREASE OF PROPHYLACTIC EFFECT OF A COMBINATION OF ATROPINE AND SODIUM FLUORIDE (i.p.) WITH TIME IN SARIN (4 LD_{50} s.c.) POISONED MICE.

Time interval between pretreatment and poisoning	10 min	30 min	60 min
Per cent mortality	20	70	100

are found. For comparison experiments were performed with the well-known antidote P2S under the same experimental conditions. 78 and 28 per cent of enzyme activity were obtained in this case. Thus both the oxime P2S and fluoride have an appreciable effect upon restoration of muscle ChE. Even brain ChE was somewhat higher than after atropine alone (Table 3).

TABLE 3. EFFECT OF SODIUM FLUORIDE AND P2S ON ChE ACTIVITY (RELATIVE ENZYME ACTIVITIES) OF BRAIN AND MUSCLE OF SARIN POISONED MICE

Tissue	Control	Sarin		
	Atropine only	Atropine	Atropine + NaF	Atropine + pralidoxime
Muscle	45 ± 2 (5) 100%	25 ± 3 (5) 56%	40 ± 5 (4) 88%	35 ± 5 (3) 78%
Brain	110 ± 4 (5) 100%	22 ± 1 (5) 20%	36 ± 6 (4) 33%	31 ± 5 (3) 28%

Effect of NaF on blood cholinesterases in Sarin poisoned dogs

Four dogs were poisoned by intravenous injection of 10–25 μg Sarin/kg body weight. The ChE activity of the red cells of the two dogs poisoned with 20 μg Sarin/kg is shown in Fig. 1. NaF was given intravenously (20 mg/kg) when the ChE activity was depressed to about 40 per cent of normal. As seen an increase of the enzyme activity to 55 and 65 per cent of normal had occurred about 45 min after the injection of NaF. Further injections of NaF produced another small increase in enzyme activity. Intravenous injection of the same amount of NaF to a normal dog had no effect on the AChE activity of the red blood cells. Thus *in vivo* reactivation of Sarin inhibited erythrocyte ChE by NaF was demonstrated.

Effect of NaF on blood pressure, heart rate and neuromuscular transmission in Sarin poisoned cats

In cats anaesthetized with sodium pentobarbital Sarin (30–50 μg/kg) produces hypotension, bradycardia, depression of neuromuscular transmission and respiration. Figure 2 shows the effect of an intravenous injection of 20 mg NaF/kg in a Sarin poisoned cat. A slight increase in blood pressure occurs and the heart rate is normal after about 3 min. The subsequent injection of atropine (1 mg/kg) establishes a normal blood pressure but has no further effect on the heart rate. In similar experiments it was shown that NaF (20 mg/kg) partially restored the ability of the gastrocnemic muscle to sustain a tetanus following indirect stimulation. This is illustrated in Fig. 3 which shows the effect on neuromuscular transmission of increasing doses of NaF (intraarterial injection close to the gastrocnemic muscle). As seen 7 mg/kg NaF almost

completely restores the ability to sustain tetanic stimulation, but a marked effect is produced by 0·7 mg/kg. This effect on neuromuscular transmission is also evident following intravenous injection of 10–20 mg/kg NaF.

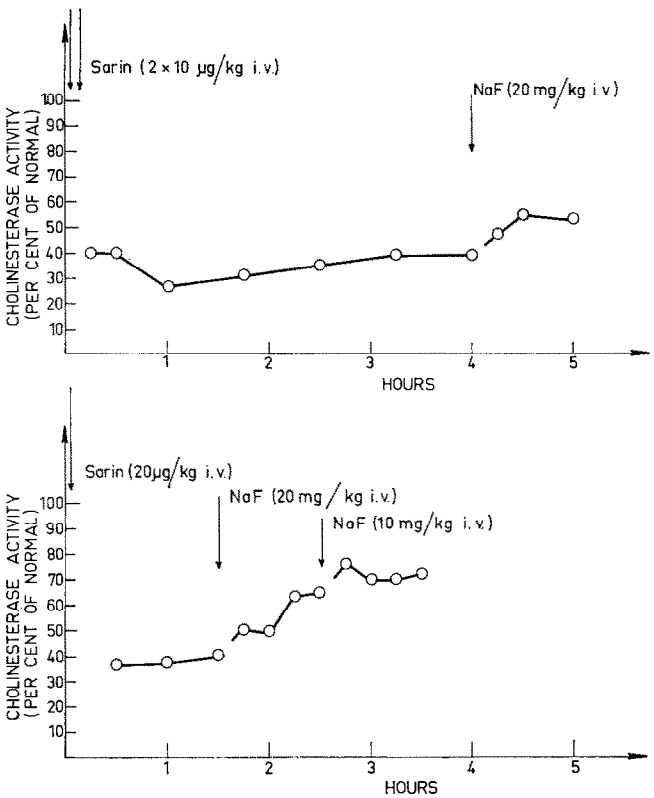

FIG. 1. The reactivating effect of intravenous injections of sodium fluoride on the ChE activity of the erythrocytes of the dog after intravenous injection of Sarin.

DISCUSSION

The relative reactivating potencies of NaF and P2S *in vitro* indicate that P2S at 37° and pH 7·4 is about 40 times more effective than NaF as a reactivator of Sarin inhibited human erythrocyte ChE (molar basis).[2] The antidote effect (as multiples of LD_{50} of Sarin) of 0·13 mmoles/kg of P2S and 0·48 mmoles/kg of NaF in atropinized mice is 6·4 and 4·5 respectively. Complete agreement between *in vitro* and *in vivo* experiments cannot be expected, especially when the experiments are performed with different animal species. Disregarding this, the experiments clearly show that a correlation exists between the relative antidote effect of the two drugs on one hand and their relative reactivating power *in vitro* on the other hand.

In the absence of atropine NaF has no antidote effect in Sarin poisoned mice while in animals poisoned with the quaternary inhibitor $^{37}S\text{-}N^+$, a drug which gives the same phosphorylated enzyme as Sarin,[9] the LD_{50} of the inhibitor is increased with a factor of 2·5. The discrepancies in the effect of NaF against Sarin and $^{37}S\text{-}N^+$ respectively

can partly be explained by differences in the distribution of the two organophosphorus compounds, as it was found that the quaternary inhibitor ^{37}S-N$^+$ penetrates poorly into the central nervous system,[8] which is not the case with Sarin. Experiments with labelled fluoride have shown also this compound to penetrate poorly into the central nervous system.[10] Therefore, NaF is a better antidote against the organophosphorus compound not passing the blood-brain barrier. Differences in the rate of inhibition, obtained *in vivo* with Sarin or ^{37}S-N$^+$ respectively, may also be of importance. It was further found that after administration of equitoxic doses of the two anticholinesterases to mice, those animals which had received ^{37}S-N$^+$ survived about 3 times longer than those having received Sarin.[11] Slower phosphorylation of blood ChE after ^{37}S-N$^+$ than after Sarin has been demonstrated *in vitro*[12] and this has also been demonstrated for closely related compounds.[13] Furthermore *in vitro* experiments with rat diaphragms showed that with equimolar doses of the compounds a maximal neuromuscular block was obtained with Sarin within 6·7 min, but with ^{37}S-N$^+$ within 16·5 min.[14] The further increase of the LD_{50} of ^{37}S-N$^+$ in the presence of atropine may be due to the fact that fluoride alone poorly counteracts muscarinic effects such as hypotension and bradycardia.

In the presence of atropine the antidotal effect of 0·48 mmoles/kg NaF is somewhat better (2·5 LD_{50}) than the effect of 0·10 mmoles/kg P2S (1·3 LD_{50})[15] in Tabun poisoning. It was shown that Tabun in water solution rapidly reacts with NaF forming a less toxic anticholinesterase,[16] dimethylamido-ethoxy-phosphoryl fluoride. This may occur also *in vivo* and partly explain the observed antidotal effect of NaF. Slow reactivation *in vitro* of Tabun inhibited ChE has been shown and can to a small extent be expected to contribute to the antidotal effect of NaF.

Determinations of ChE activities in brain from atropinized Sarin poisoned mice showed that after pretreatment with either sodium fluoride or P2S somewhat higher ChE activities were obtained than after pretreatment with atropine only. The results with P2S are in agreement with earlier reports in the literature.[19] In skeletal muscle both drugs caused a substantially higher ChE activity, possibly due to protection of the enzyme before inhibition and/or reactivation of the inhibited enzyme. This latter effect was demonstrated *in vivo* by the dog experiments, where each administration of NaF was followed by an increase in enzyme activity.

The effects of fluoride on the toxic symptoms produced by Sarin in the anaesthetized cat also closely resembled the effects produced by P2S. The neuromuscular block is rather effectively reversed by 10 to 20 mg/kg NaF intravenously. Besides reactivation of the inhibited enzyme the fluoride ion also has the ability to increase the sensitivity of the motor end plate to acetylcholine.[20] However, this anticurare effect would increase the neuromuscular block produced by anticholinesterase rather than reverse the block. Therefore it seems likely that the principal action of sodium fluoride in Sarin poisoning is due to its reactivating power. On the other hand the reversal of the neuromuscular block is more rapid than would be expected if reactivation was the only mechanism, a discrepancy that is also evident with other reactivators such as P2S and TMB-4.[21] The fact that the bradycardia produced by Sarin is not reversed as rapidly by fluoride as by P2S can probably be explained by the atropine-like properties of P2S.

It thus seems justified to conclude that the antidotal effect of NaF in Sarin poisoning is mainly due to the reactivating effect of the drug. In Tabun poisoning reactivation

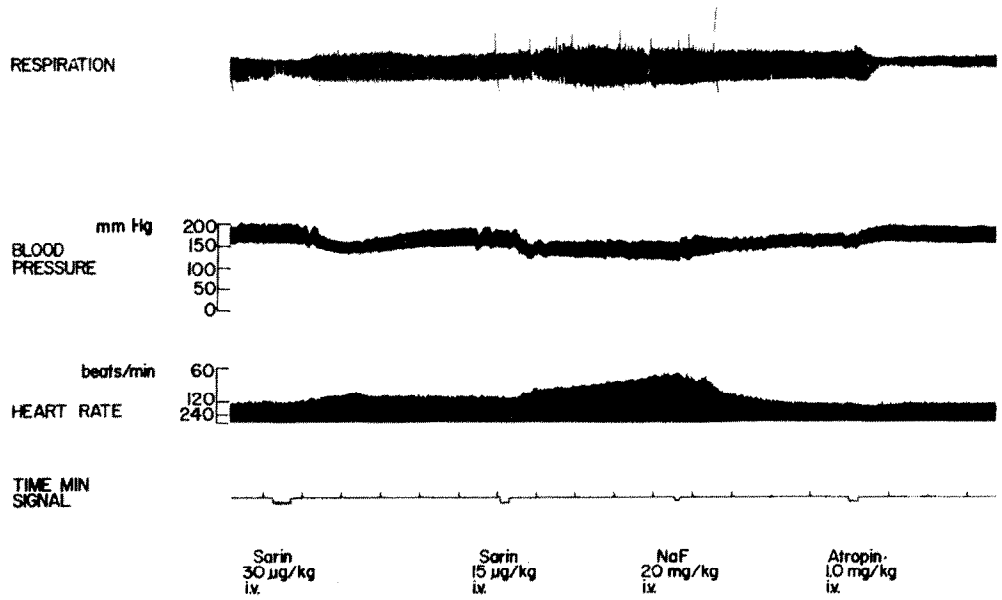

FIG. 2. Effects of intravenous injection of sodium fluoride and atropine on respiration, blood pressure and heart rate after Sarin poisoning Cat 3·2 kg Sodium pentobarbital.

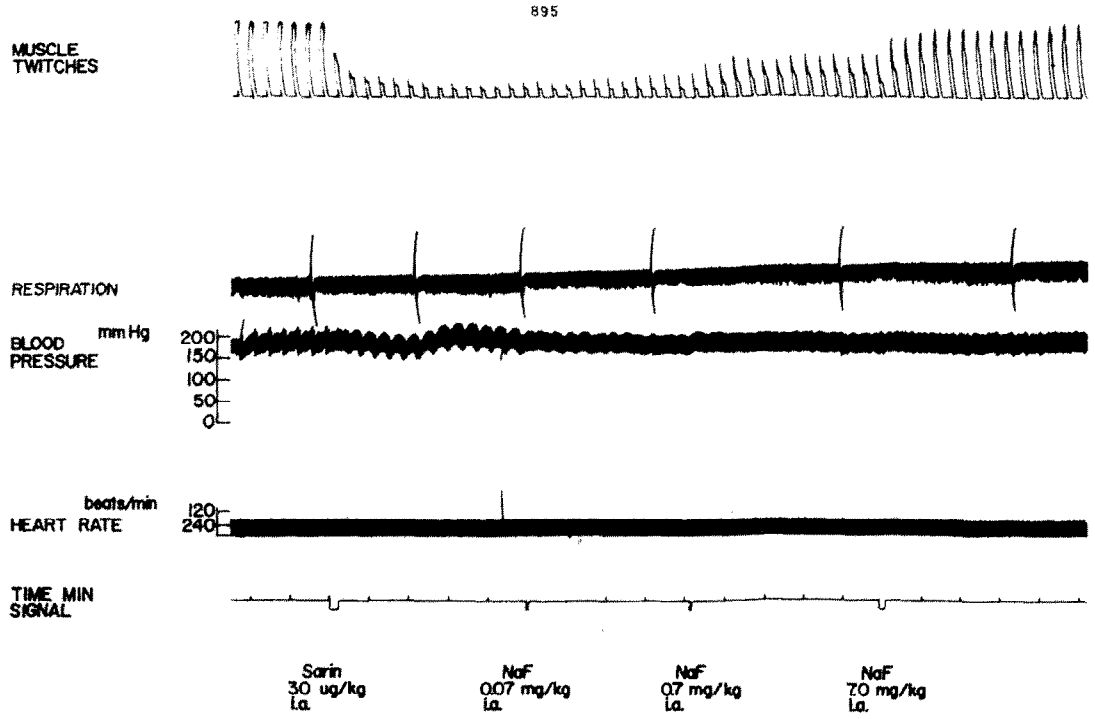

FIG. 3. Effects of intraarterial injections of sodium fluoride on the function of the gastrocnemic muscle (tetanic stimulation of the sciatic nerve) after intraarterial injection of Sarin. The injections are made close to the aortic bifurcation. Cat 3·4 kg Sodium pentobarbital.

is slow, but a reaction between NaF and Tabun, resulting in a less potent anticholinesterase, contributed to the antidotal effect. The antidotal effect was highest in $^{37}S\text{-}N^{+}$ poisoning, mainly because no central effects had to be counteracted. However, the general antidotal effect of NaF is weak and in view of the relatively high toxicity of sodium fluoride the compound is probably not suitable for practical purposes

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