found a comparable phenomenon in the subcellular distribution of phenothiazines in the rabbit platelets, where the main part of chlorpromazine sulphoxide remained in the supernatant while chlorpromazine and desmonomethylchlorpromazine were bound to particulate fractions.[7] Both these phenomena correlate to the low lipid-solubility of chlorpromazine sulphoxide.[7]

The quaternary *N*-hydroxyethylpromethazine, being poorly lipid-soluble,[7] is taken up by red cells even less than chlorpromazine sulphoxide[2, 3] but its protein binding was relatively high. Therefore, the protein binding of the drugs cannot be solely correlated to their lipid-solubility but other physicochemical properties of the drugs are also of importance.[8]

Acknowledgement—The authors wish to acknowledge Prof. N.-E. Saris for his valuable advice.

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Biochemical Pharmacology, Vol. 16, pp. 2435–2438. Pergamon Press Ltd. 1967. Printed in Great Britain

Blood concentrations of *N,N'*-trimethylenebis(pyridinium-4-aldoxime) (TMB-4) and *N,N'*-oxydimethlenebis (pyridinium-4-aldoxime) (toxogonin) after intravenous and intramuscular administration in the dog

(*Received* 7 *June* 1967; *accepted* 21 *August* 1967)

BLOOD concentrations of *N,N'*-trimethylenebis (pyridinium-4-aldoxime) (TMB-4) and *N,N'*-oxydimethylenebis (pyridinium-4-aldoxime) (Toxogonin, Lü H6) at various times after intravenous and intramuscular administration of oximes (20 mg/kg) have been estimated in dogs anaesthetized with chloralose. The half-life was 28·3 min for TMB-4 and 19·9 min for Toxogonin after i.v. injection. The rates of absorption of either oxime were equal and the maximum blood concentrations were reached within 15 min after intramuscular injection.

In a series of papers,[1, 2, 3] Erdmann and coworkers have tried to show that Toxogonin is in many respects superior to pralidoxime (2-PAM) and TMB-4 as an antidote against organophosphorus anticholinesterases. However, Heilbronn and Tolagen[4] have found that the cholinesterase reactivating power of Toxogonin in experimental sarin or tabun poisoning is comparable to that of TMB-4. Hobbiger and Vojvodić,[5] also, could not find any substantial difference in reactivating and antidotal effects of TMB-4 and Toxogonin in mice and rats poisoned with several organophosphorus compounds.

The present experiments were carried out in order to compare the concentrations of TMB-4 and Toxogonin in the blood, following i.v. and intramuscular injection of these oximes in the dog. Previous clinical and experimental studies[6, 7, 8] have shown that TMB-4 relatively quickly disappears from the blood which is, undoubtedly, a serious disadvantage of this oxime, especially in the case of poisoning with organophosphorus compounds with a long persistence in the body.

METHODS AND MATERIALS

The experiments were performed using dogs anaesthetized with chloralose (0·1 g/kg, i.v.). Fresh solutions of TMB-4 dichloride (m.p. 222°) and Toxogonin dichloride (m.p. 220°) in distilled water were injected i.v. (v. saphena) or intramuscularly (gluteal region). The fixed dose of 20 mg/kg of oximes was used in all experiments. For each oxime and either route of administration at least 3 dogs were used.

The concentrations of oximes were measured in the whole blood, since Erdmann and Engelhard[2] have shown that Toxogonin readily penetrates the membrane of the red blood cells with the concentration of oxime in the cells being almost the same as in the blood plasma. The blood sample (2 ml) were taken from the femoral artery and collected in heparinized test tubes. Blood oxime levels were determined by the spectrophotometric method (Beckmann DU Spectrophotometer) of Creasy and Green.[9] The test solutions needed for this procedure were made alkaline, and the optical densities were measured at 345 mμ for estimating TMB-4 and at 350 mμ for estimating Toxogonin. The oxime concentration was read from a calibration curve after correction for the absorption of blood at these wave-lengths. The calibration curve was made in each experiment. Since Toxogonin was found to be considerably less stable in alkaline medium than TMB-4,[5] all readings were done within 30 sec after adding 20% solution of NaOH.

RESULTS

The concentrations of TMB-4 and Toxogonin in whole blood, following the i.v. injection of 20 mg/kg oximes, in the dog, are shown in Fig. 1. During the first 10 min after injection the blood concentration of either oxime falls quickly, due to rapid diffusion of oximes from the blood into the

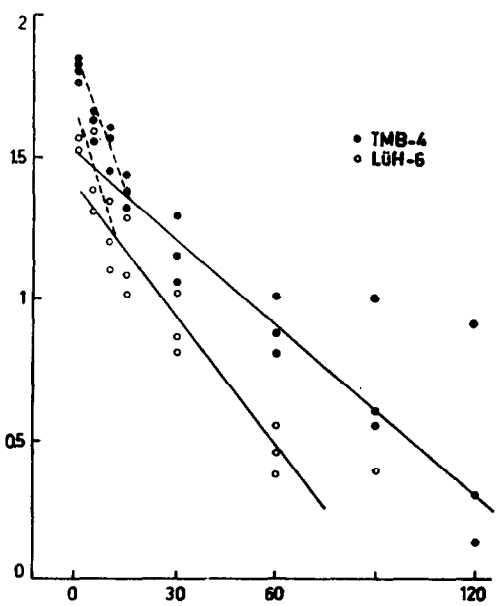

FIG. 1. Blood concentrations of TMB-4 and Toxogonin in dogs after i.v. administration of 20 mg/kg of oximes. *Ordinate:* Log concentration (μg/ml). *Abscissa:* Time (in minutes) after injection of oximes.

tissues. At the end of this period the relative equilibrium of oximes between blood and tissues is established, and the effect of elimination begins to prevail. The rate of disappearance of oximes from the blood is then linear with the time and slower than during the initial period.

The regression lines for the decrease of blood oxime concentrations during the late stage correspond to equations:

$$\text{Log C } (\mu\text{g/ml TMB-4}) = 1{\cdot}5078 - 0{\cdot}0106\ t$$
$$\text{Log C } (\mu\text{g/ml Lü H6}) = 1{\cdot}3641 - 0{\cdot}0151\ t$$

From these equations the log concentration at any time (t) can be calculated for either oxime (ten minutes after injection of oxime). The half-life of oximes, calculated from regression lines, was 28·3 min for TMB-4 and 19·9 min for Toxogonin.

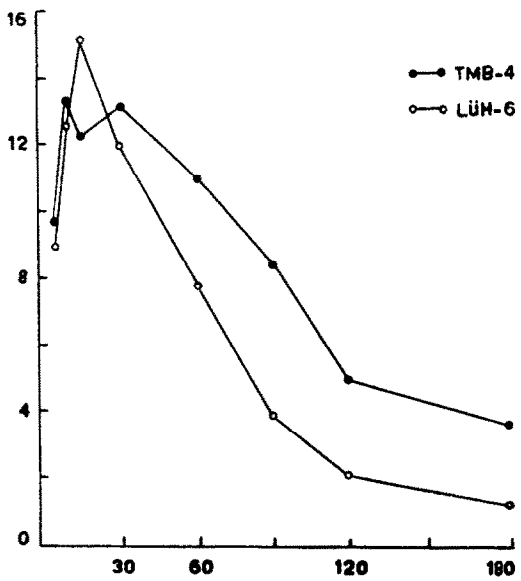

FIG. 2. Average blood concentrations of TMB-4 and Toxogonin in dogs after intramuscular injection of 20 mg/kg of oximes. *Ordinate:* Concentration of oximes (μg/ml). *Abscissa:* Time (in minutes) after injection of oximes.

Fig. 2 shows the average concentrations of TMB-4 and Toxogonin in the blood of dogs at different intervals after the intramuscular injection of 20 mg/kg of oxime. As can be seen, the maximum blood concentrations of either oxime was reached within 15 min after injection. For Toxogonin the maximum blood level was about 15 μg/ml, and about 13 μg/ml for TMB-4. This difference is not statistically significant ($P > 0{\cdot}05$). After reaching the maximum, the concentration of TMB-4 in the blood decreases more slowly than the concentration of Toxogonin. Three hours after injection, the blood level of oximes was 4 μg/ml TMB-4 and 1–2 μg/ml Toxogonin, respectively.

DISCUSSION

The results of these experiments show that the intravenous injection of 20 mg/kg (0·056 mM/kg) of TMB-4 and Toxogonin produce relatively high concentrations of the oximes in the blood of the anaesthetized dog. Immediately after injection, the concentration of TMB-4 in the whole blood reaches a higher level than that obtained with Toxogonin. The concentration of TMB-4 in the blood was always higher than that of Toxogonin, during the experimental period of three hours.

The half-life for TMB-4 (28·3 min) corresponds to that previously reported in rats[7] and rabbits,[8] after i.v. injection of 15 and 18 mg/kg of TMB-4, respectively. The half-life for Toxogonin in the anaesthetized dog (20 mg/kg, i.v.) was markedly shorter than that found by Erdmann and Engelhard[2] in the anaesthetized cat following i.v. injection of 50–100 mg/kg. The reason for this disagreement

is not clear, but it might be probably explained by the difference in the methods used for the measurement of oximes in the blood.[10] The species differences might be of some importance as well.[11]

The intramuscular administration of oximes gives a rapid absorption from the site of injection but in this case, too, the blood level of TMB-4 was somewhat higher during the 3-hr period than that of Toxogonin. At the end of the experimental period the mean concentration of Toxogonin was about 1–2 μg/ml while the concentration of TMB-4 was twice this amount. If the concentration of 0·1–0·2 μg/ml of Toxogonin is regarded adequate for a therapeutic effect in organophosphorus compounds poisoning [3] the blood concentration of 1–2 μg/ml of Toxogonin must be considered as a very effective one. Since there is no substantial difference in reactivating potencies between TMB-4 and Toxogonin,[5, 12] it can be expected, with a high degree of probability, that the therapeutic effectiveness of this oxime will be at least equal if not greater than that of Toxogonin.

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Biochemical Pharmacology, Vol. 16, pp. 2438–2441. Pergamon Press Ltd. 1967. Printed in Great Britain

Choline acetyltransferase inhibitors: a group of styryl-pyridine analogs

(*Received* 1 *June* 1967; *accepted* 28 *June* 1967)

A GROUP of styryl-pyridine analogs that inhibit choline acetyltransferase have been studied. The most potent of these was hexamethylene-1-4-(1-naphthylvinyl)-pyridinium-6-trimethylammonium dibromide with an I_{50} value of 9×10^{-7} M. The anti-choline acetylase activity appears to be associated with the large conjugated, planar, lipophilic moiety. The trimethylammoniumalkane appendage does not seem to be a qualitatively critical structure and contributes less to choline acetylase inhibitory activity than to cholinesterase inhibition. The most specific inhibitor for choline acetylase is 4-(1-naphthylvinyl)-pyridine with an I_{50} of 3×10^{-5} M, which inhibits cholinesterase only at high concentrations.

Choline acetyltransferase (choline acetylase; acetyl-CoA: choline O-acetyltransferase, EC 2.3.1.6; ChA) is responsible for the synthesis of acetylcholine (ACh) in nervous and other tissues. A variety of types of compounds have been reported to be weak inhibitors of this enzyme (reviewed by Nachmansohn[1]). Few of these have I_{50} values below 10^{-3} M. The availability of a potent and specific ChA inhibitor would be of great interest in further elucidating the role of this enzyme in brain and