An oxime analogue of atropine—some pharmacological observations

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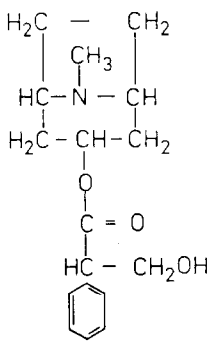
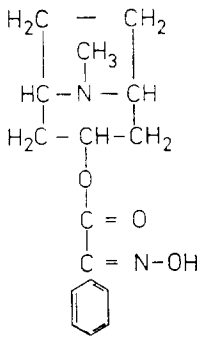
IN RECENT years certain oximes have been found to be valuable adjuncts to atropine in the treatment of organophosphorus anticholinesterase poisoning. These oximes reactivate the phosphorylated cholinesterase produced by the poison thus providing a causal therapy, while atropine restores the function of autonomic effectors and of the respiratory centre.

An oxime analogue of atropine might combine the parasympatholytic and reactivating effects in one drug. For the first time a substance of this kind—phenylglyoxylic acid tropylester oxime (here called PGTO)—has been synthesized by Tammelin and Flormark[1] and it seemed interesting to study its antidotal effect on anticholinesterase poisoning.



Atropine



Phenylglyoxylic acid tropylester oxime (PGTO)

PGTO was found to be about four times more toxic than N-methylpyridinium-2-aldoxime methane sulphonate, P2S, (LD_{50} in mice: 37 ± 0·12 mg per kg bodyweight by intraperitoneal administration). On acetylcholine stimulated guinea pig ileum in Tyrode solution an antispasmodic effect was obtained, which was found to be ten times less than that of atropine. Using the technique of van Rossum and Ariens[2] on rat jejunum a parasympatholytic action was confirmed. In survival experiments in mice 25 mg per kg body-weight of PGTO did not save the animals against 1·5 times the LD_{50}-dose of ω-dimethylaminoethylthio*iso*propoxymethylphosphine oxide, a potent cholinesterase-inhibitor, (30 mg of P2S per kg bodyweight gives complete protection against twice the LD_{50}-dose of the cholinesterase-inhibitor used).

In the isolated rat diaphragm a depression of the twitch height was observed at concentrations down to 5×10^{-5} M of PGTO. The muscle was stimulated through the phrenic nerve by supra-maximal stimulations for 4 sec every 30 sec, alternating 30 and 50 imp/sec with a duration of 1·2 msec. On this preparation PGTO in a molar concentration of 5×10^{-6} did not reverse the neuromuscular block produced by the hydrogen oxalate of ω-dimethylaminoethylthio*iso*propoxy-methylphosphine oxide (5×10^{-7} M) while the muscle function returns to normal when adding 5×10^{-6} M of P2S.

In the anaesthetized cat the influence of PGTO on the toxic effects of the methiodide of ω-dimethylaminoethylthio*iso*propoxy-methylphosphine oxide was studied. A parasympatholytic effect was observed with 5 mg of PGTO per kg bodyweight intravenously. The blood pressure was normalized, the pulse rate hastened and respiration was stimulated. The effects were shortlasting. No action was observed on the blocked muscle function revealed by stimulation of the sciatic nerve to gastrocnemius.

Though many compounds with the —CO.CH:NOH— group show reactivating properties[3, 1] no such effect could be obtained with PGTO in animal experiments. With the technique described by Enander[5] the lack of reactivating properties *in vitro* was also proved with 5×10^{-3} M of PGTO for 45 min using methyl*iso*propoxyphosphorylated cholinesterase from human erythrocytes.

Thus the only action found was a parasympatholytic effect, ten times less than that of atropine. No reactivating properties were detected with this substance.

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N-hydroxylation of carcinogenic amines *in vivo* and *in vitro* with liver microsomes

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THE N-oxidation of aromatic amino compounds was first demonstrated *in vivo* by Kiese.[1] Cramer *et al.*[2] isolated N-hydroxy-2-acetylaminofluorene from the urine of rats fed with 2-acetylaminofluorene, and Wyatt *et al.*[3] found N-hydroxy-4-acetylaminodiphenyl as an urinary metabolite of 4-acetylaminodiphenyl. Troll and Nelson[4] detected a derivative of 2-naphthylhydroxylamine in the urine of dogs and patients exposed to 2-naphthylamine.

In the organism, the reactive N-oxidation products lead to the formation of methaemoglobin and other toxic symptoms. Formation of methaemoglobin following application of amino compounds gives strong evidence that the formation of amines to hydroxylamino and nitrosocompounds has occurred in the body.[5]

We have shown that N-hydroxylation of aromatic amines and N-alkylamines is catalysed *in vitro*, in the presence of TPNH and oxygen, by isolated liver microsomes.[6] N-Hydroxylation of 2-aminofluorene[7] and 2-naphthylamine[8] by such a system has been reported. The combination of hydroxylamino and nitroso compounds with proteins may produce antigenic substances.[9] It seems, that the reactive N-oxidation products play important roles in the carcinogenicity of aromatic amines and their derivatives. Therefore, we have investigated N-hydroxylation of several other carcinogenic amines *in vivo* and *in vitro*.

METHODS

Cats were injected intraperitoneally with the amines (0·5 m-mole/kg), dissolved in a mixture of 10% gum arabic, 5% 1:2-propylene glycol and 85% NaCl (0·9%). Blood samples were withdrawn from the carotid artery. Methaemoglobin was estimated, after haemolysis and addition of cyanide, at 550 mμ. Microsomes were prepared from homogenates of rat livers in a phosphate buffer (0·1 M; pH 7·4) by centrifugation for 1 hr at 78,000 × *g* and washed twice with the phosphate buffer. Incubation mixtures were prepared as indicated in Fig. 2. 4-Nitrosodiphenyl was synthesized from 4-aminodiphenyl by oxidation.[10] Hydroxylamino and nitroso compounds were estimated together, after extraction with CCl_4 in the presence of Fe^{3+} and removing amines, by comparing their adsorption spectra with that of synthetic products or, alternatively, by spectrophotometric analysis of the compounds resulting from diazotization and coupling.[11]

RESULTS AND DISCUSSION

Following injections of carcinogenic amines, methaemoglobin was found in the cat, a fact which can be explained by N-oxidation (Fig. 1). It should be mentioned here that 4-aminostilbene and 2-aminofluorene are effective only when dissolved in 1:2-propylene glycol, but haemoglobin is not oxidized by injections of 1:2-propylene glycol alone. The rates of haemoglobin oxidation, however, cannot accurately be compared without taking into consideration the significant differences in blood amine concentrations.