Biochemical basis for the toxic effects of triethyl lead

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Galzigna, L., Ferraro, M. V., Manani, G., and Viola, A. (1973). *British Journal of Industrial Medicine*, 30, 129-133. **Biochemical basis for the toxic effects of triethyl lead.** The effects of triethyl lead (PbEt_3) have been studied *in vitro* on the cholinesterase activity of rat diaphragm and *in vivo* on serum cholinesterase in the dog. PbEt_3 dramatically increases the duration of succinylcholine-induced myoneural block and pyridine-2-aldoxime methiodide (PAM) is able to counteract both cholinesterase inhibition and the effects on neuromuscular transmission. On the other hand, PbEt_3 catalyses the rearrangement of catecholamines to aminochromes *in vitro* and inhibits catecholamine effect on smooth muscle contraction. The toxicity of PbEt_3 and particularly its action on the central nervous system can be explained by a combination of effects which might result from an upset of cholinergic and adrenergic central pathways due to the formation of endogenous psychotogenic complexes.

In the last few years we have been concerned with the problem of the biochemical basis of the toxicity of alkyl derivatives of lead, particularly by the interference of such compounds with normal metabolic processes.

First we analysed in some detail the effect of tetraethyl lead (PbEt_4) on the metabolism of the hydroxylated derivatives of tryptophan (Corsi, Galzigna, and Brugnone, 1964). Our results demonstrated that biosynthesis of serotonin is somewhat affected whereas its catabolism is not influenced. Subsequently our attention was focused on the effects of PbEt_4 and PbEt_3 , its active metabolite (Cremer, 1965), on cholinesterase activity. An inhibitory effect of PbEt_3 on purified serum cholinesterase was shown *in vitro* and PAM was able to restore the activity of the inhibited enzyme (Galzigna, Corsi, Saia, and Rizzoli, 1969). These results were questioned on the basis of data showing that no inhibition of blood cholinesterases is observable when a concentration of PbEt_3 of the same order as

the toxic dose is used (Chiesura, 1970). We must specify that we did not intend to propose a method for biochemical diagnosis of the intoxication but only to build up a phenomenological model which might account for the toxic effects of PbEt_3 . We based our assumptions on a localized effect of PbEt_3 at the level of the central nervous system (CNS) (Cremer, 1965) in the light of the particular importance of the aspecific cholinesterase of CNS considered as a barrier for the neurotransmitter acetylcholine (Galzigna, 1970). On the other hand, we wish to emphasize that all tissues are a particularly heterogeneous system and the chemical potential of any toxic compound reaches its highest value at the level of specific structures within the tissue so that the concentration in a target area must be much higher than the concentration referred to the whole tissue or the whole body.

In this paper we present further data on the inhibitory effects of PbEt_3 on cholinesterase both *in vitro* and *in vivo* and on its interference with the mechanisms of the succinylcholine-induced depolarizing block of cholinergic transmission. These data will be discussed together with the effects of

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PbEt₃ on adrenergic mechanisms on the basis of our recent studies on the biochemistry of psychotic reactions (Galzigna, 1972).

Materials and methods

Phrenic-diaphragm preparations obtained from 10 Wistar strain adult male rats were used as a source of crude acetylcholinesterase for the *in vitro* experiments. The enzymatic activity was detected with acetylthiocholine (Fluka, Buchs) substrate using rat diaphragm homogenates in 0.25 M sucrose as enzyme and dithio-bisnitrobenzoic acid (Fluka, Buchs) as a chromogen, according to Ellman, Courtney, Andres, and Featherstone, (1961).

PbEt₃ chloride (Sloi, Trento) free from inorganic lead was dissolved in double distilled water and used for both *in vitro* experiments and intravenous administration to 10 adult male dogs (6-12 kg body weight). The effect of PbEt₃ (6 mg/kg) was checked on serum cholinesterase activity followed at intervals after its administration. The interference of PbEt₃ on a depolarizing myoneural block induced by succinylcholine (Sincurine, Wellcome) was studied by an electromyographic (EMG) technique already described (Galzigna *et al.*, 1972) and the protective action of PAM (Farmitalia, Milan) on both cholinesterase inhibition and neuromuscular function was demonstrated.

The chemical interaction between PbEt₃ and norepinephrine (or dopamine) (Calbiochem, Los Angeles) in a dioxane-water system was documented according to the procedure used in the previous studies (Galzigna, 1970; 1972) and the interference of PbEt₃ on catecholamine action was assayed with the isolated rabbit ileum technique (Galzigna *et al.*, 1972). Three domestic rabbits were used and the contractile activity of the ileum dipped in 75 ml of Tyrode's solution was followed with a kymograph at 37°C.

Further details of the experimental conditions are given in the captions to the figures.

Results

Figure 1 shows the effect of different concentrations of PbEt₃ *in vitro* on acetylcholinesterase activity of rat diaphragm with two concentrations of substrate acetylthiocholine. The inhibition is non-competitive, as proved by plotting Fig. 1 results as the reciprocal of the velocity of acetylcholine hydrolysis versus the concentration of the inhibitor (Webb, 1966). The highest inhibition obtained corresponds to about 25% of the normal activity and as we proved (Galzigna *et al.*, 1972) that the usual inhibitors of aspecific (or pseudo-) cholinesterase are able to induce a maximal inhibition of 25-30% on the true cholinesterase activity of rat diaphragm preparation, it seems that PbEt₃ has this characteristic in common with aspecific cholinesterase inhibitors.

An apparent K_i value of the order of 10^{-3} M for the crude enzyme, much higher than the value obtained for the purified enzyme (Galzigna *et al.*, 1969), can be calculated.

FIG. 1. Inhibitory effect of different concentrations of PbEt₃ on acetylcholinesterase activity of rat phrenic-diaphragm homogenates. An activity of 100 corresponds to 0.01 μ Mole of acetylthiocholine hydrolysed per minute per mg of protein of the homogenate. Colorimetric values are obtained with a Beckman DU spectrophotometer at 25°C and 412 nm. A calibration curve with N-acetyl-L-cysteine (Erba, Milan) is used. The two levels of 0.26 M acetylthiocholine are 0.4 and 0.8 ml in a final volume of 4 ml of incubation mixture. Each value is an average of 10 experiments.

Figure 2 shows the percent inhibition of serum cholinesterase assayed in blood taken at different times after administration of PbEt₃ to dogs. Maximum inhibition is observed 15 minutes and full

FIG. 2. Inhibition of serum cholinesterase after PbEt₃ (6 mg/kg) given to dogs by fast flowing infusion, and the protective effect of PAM (36 mg/kg). The values are an average of 10 experiments (5 with PbEt₃ alone and 5 with PbEt₃ and PAM). A normal activity corresponds to 0.08 μ Moles of acetylthiocholine hydrolysed per minute per ml of serum.

recovery 45 minutes after administration. The administration of PAM brings back almost immediately the activity of serum cholinesterase at normal values.

The effect of PbEt_3 on a succinylcholine induced depolarizing block is shown in the EMG tracings (Fig. 3). A complete recovery of transmission does not occur until 66 minutes whereas succinylcholine

FIG. 3. Typical EMG tracing obtained after single-tetanic-single sequences of stimulation. Succinylcholine (0.05 mg/kg) is given 10 minutes after PbEt_3 (8 mg/kg). The control (a), showing normal transmission, is followed by a tracing taken 11 minutes after succinylcholine administration (b).

The recovery is slow and gradual and almost normal neurotransmission (h) returns after 66 minutes.

Without PbEt_3 the recovery begins 4 minutes after succinylcholine and is complete by 6 minutes. If PAM (36 mg/kg) is given together with succinylcholine 10 minutes after PbEt_3 the recovery begins within 2 minutes and is complete by 5 minutes. The EMG changes are in any case typical of a depolarizing block.

alone, in the same conditions, induces a block lasting only 6 minutes. This is explained by competition between PbEt_3 and succinylcholine for serum cholinesterase. The presence of PbEt_3 allows a very long duration of succinylcholine action and PAM is able to abolish completely such an effect.

The recording of electroencephalographic activity after PbEt_3 was used to monitor the subcortical effects of PbEt_3 at the CNS level and a complete discussion of this aspect is given elsewhere (Manani *et al.*, 1972).

Figure 4 shows the absorption spectrum of norepinephrine in a dioxane-tris mixture taken 1 hour after the addition of PbEt_3 . The typical absorption peaks of aminochromes are present in the PbEt_3 treated sample, demonstrating the catalytic effect of PbEt_3 on the cyclization of norepinephrine. Inorganic lead does not induce a similar effect, probably because it is insoluble in the mixture. This has a low dielectric constant and is taken as a model of the physicochemical features of the CNS milieu (Galzigna, 1970).

The ability of PbEt_3 to antagonize the effect of norepinephrine *in vivo* is demonstrated in Fig. 5 in which both the relaxation induced by norepinephrine on rabbit ileum contraction and the recovery of the contractile activity due to PbEt_3 are shown. PbEt_3 alone at the same concentration was without any effect on normal contractile activity.

Discussion

The relationship between enzymatic inhibition induced by a compound and its toxic power has been extensively studied (Webb, 1966) and some analytical expressions which quantitatively define such a

FIG. 4. Absorption spectra of 0.0125 M norepinephrine (NE) dissolved in a mixture (v/v) of 0.25 M tris-HCl, pH 7.8, and dioxane (1:1) taken 1 hour after addition of 8.5×10^{-3} M PbEt_3 .

FIG. 5. Recording of rabbit ileum contraction. The tracing shows after normal contractile activity the relaxation by norepinephrine (NE) and the contraction induced by PbEt_3 .

relationship have been formulated. We summarize such expressions by the formula:

$$(I)_t = K_i/C$$

where $(I)_t$ is the toxic dose of a compound, K_i is the constant for the inhibition of an enzymatic reaction induced by the compound, and C is a constant depending on the kinetic constants relative to the deactivation of the compound *in vivo*.

The most common situation occurs when C is large and therefore $(I)_t$ is much lower than K_i .

The present data are in agreement with our previous results on the inhibition of purified cholinesterase and the reactivating effect of PAM. The EMG results are easily understood in terms of blood cholinesterase inhibition induced by PbEt_3 which in this way is able to increase the duration of the succinylcholine dependent neuromuscular block. At CNS level an inhibition of the aspecific cholinesterase may result in a sort of acetylcholine overflow which would be very dangerous if it occurred at the same time as aminochromes formation (Galzigna, 1970).

In fact we have shown that PbEt_3 is able to catalyse *in vitro* the reaction:

The combined effect of acetylcholine 'leaks' from cholinergic synapses and aminochrome formation might lead to the presence of psychotogenic acetylcholine-aminochrome complexes which have been indicated as the possible biochemical cause for the

onset of some types of psychotic disturbances (Galzigna, 1970). In fact a true psychotogenic effect of aminochromes has been found only when they are combined with acetylcholine to form molecular complexes (Galzigna and Rizzoli, 1970).

The complexity of the action of alkylated derivatives of lead can be well explained by their multimodal effect on the CNS and particularly by the interference with cholinergic, adrenergic, and serotonergic processes. We think, however, that the psychotic symptoms which are characteristic of PbEt_4 intoxication can be rationalized on the basis of the following scheme:

The transformation of PbEt_4 into PbEt_3 has been shown by Cremer (1965). The toxic action of PbEt_3 might be related mainly to a transformation of norepinephrine into an aminochrome which is stabilized by acetylcholine leaking from cholinergic neurones after inhibition of pseudocholinesterase. These combined effects result in the formation of a psychotogenic complex particularly active at the central nervous system level.

The biochemical basis of treatment with chlorpromazine, which has recently been demonstrated as a trapping agent of aminochromes capable of successful competition with acetylcholine (Galzigna, 1972), may now be found. On the other hand, PAM should reactivate the blocked cholinesterase and in such a way stop the possible acetylcholine leaks.

All the present experimental findings await future

clinical studies which will allow the proposed model to be either confirmed or rejected.

We wish to thank Professor M. Crepet and A. Gasparetto for their encouragement, and Drs. F. Battocchio and S. Mammano for technical collaboration.

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Received for publication May 30, 1972.

Accepted for publication August 19, 1972.