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SHORT COMMUNICATIONS

Affinity and carbamylation rate constants of propoxur in reaction with erythrocyte and serum cholinesterase

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Propoxur (*o*-isopropoxyphenyl *N*-methylcarbamate) is a commonly used pesticide. Studies with human volunteers have shown that this compound produces a rapid inhibition of erythrocyte acetylcholinesterase but has little effect on the activity of serum cholinesterase [1]. This difference in inhibitory potency has been attributed to a greater affinity of propoxur for the red cell enzyme, since subsequent work *in vitro* [1] has shown that the I_{50} is $4{\cdot}6 \times 10^{-7}$ and $7{\cdot}3 \times 10^{-5}$ M for the erythrocyte and serum enzymes respectively. However, the I_{50} value does not necessarily give a measure of affinity, as can be seen from the following relationship: $I_{50} = 0{\cdot}69/tk_i$ where $k_i = k_c/K_a$ [2]. The I_{50}, like the bimolecular rate constant k_i, is determined by a carbamylation rate constant (k_c) and an affinity constant (K_a) [2]. If these constants can be measured, as was done with the organophosphate diisopropylfluorophosphate (DFP), for acetylcholinesterase and serum cholinesterase [3], then the contribution of affinity may be more accurately assessed. With DFP, the greater inhibition of the serum enzyme resulted directly from better affinity rather than from an increase in phosphorylation rate.

Employing methods described previously [3, 4], the carbamylation and affinity constants of propoxur have now been determined for commercially available bovine erythrocyte acetylcholinesterase (EC 3.1.1.7) and horse serum cholinesterase (EC 3.1.1.8). Figure 1 shows the log v vs time plots. Each inhibitor concentration yields an inhibition rate which is used to construct the plot shown in Fig. 2. In contrast to DFP, the inhibitory effect of propoxur on acetylcholinesterase is greater because its carbamylation rate is higher: 70·5 min^{-1} and 5·8 min^{-1} for the erythrocyte and serum enzymes respectively. The affinity of propoxur for acetylcholinesterase ($1{\cdot}54 \times 10^{-3}$ M) is similar to that for serum cholinesterase ($1{\cdot}44 \times 10^{-3}$ M).

Although the enzyme sources used in the present study are from different species, it is probable that the inhibitory effects noted with propoxur in the human studies can also be attributed to variations in carbamylation rate rather than affinity. This is consistent with the finding that human and horse serum enzymes are similar kinetically [5] and that acetylcholinesterases of various mammalian sources

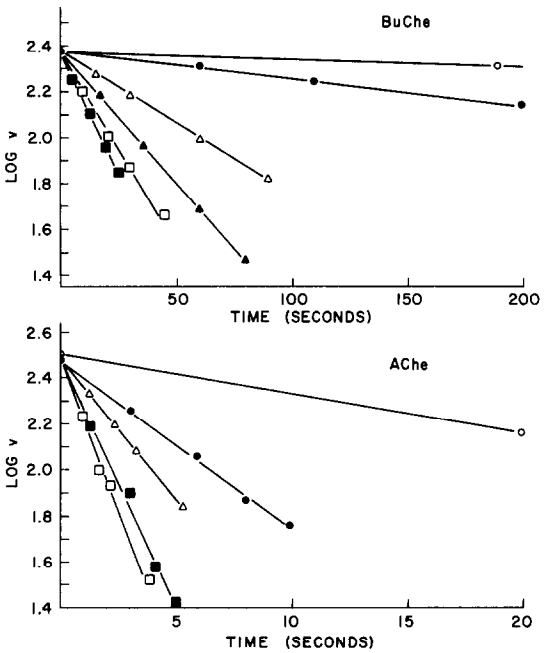

Fig. 1. Log v versus time plots for inhibition of serum cholinesterase (BuChE) and erythrocyte acetylcholinesterase (AChE) by propoxur. Inhibitor concentrations are as follows: BuChE: ○, $1 + 10^{-5}$ M; ●, 5×10^{-5} M; △, $2{\cdot}5 \times 10^{-4}$ M; ▲, 6×10^{-4} M; □, 1×10^{-3} M; ■, $1{\cdot}5 \times 10^{-3}$ M. AChE: ○, 5×10^{-5} M; ●, $2{\cdot}5 \times 10^{-4}$ M; △, 5×10^{-4} M; ■, 1×10^{-3} M; □, $1{\cdot}5 \times 10^{-3}$ M. Each inhibitor concentration yields a value of ρ or 2·3 Δ log $v/\Delta t$. Inhibition occurred after mixing enzyme and inhibitor in an inhibition reaction vessel [3] at 25°, pH 7·6. Residual enzyme activity was measured in a Radiometer pH-stat equipped with a thermostatted reaction vessel containing 3×10^{-3} M acetylcholine chloride for the AChE assay or 1×10^{-2} M butyrylcholine iodide for the BuChE assay.

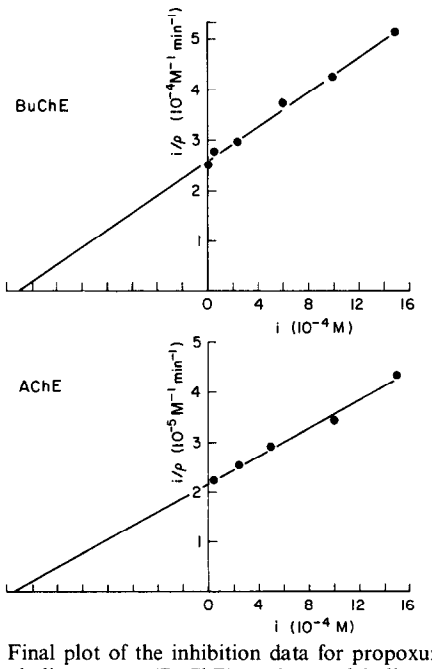

Fig. 2. Final plot of the inhibition data for propoxur and serum cholinesterase (BuChE) and acetylcholinesterase (AChE). Each ρ value produces a data point, where $i/p = i/k_c + K_a/k_c$. For BuChE, $K_a = 1{\cdot}44 \times 10^{-3} \pm 0{\cdot}17$ M; $k_c = 5{\cdot}76 \pm 0{\cdot}19$ min^{-1}; $k_i = 4{\cdot}0 \times 10^3$ M^{-1} min^{-1}. For AChE, $K_a = 1{\cdot}54 \times 10^{-3} \pm 0{\cdot}19$ M; $k_c = 70{\cdot}52 \pm 3{\cdot}77$ min^{-1} and $k_i = 4{\cdot}58 \times 10^4$ M^{-1} min^{-1}.

do not exhibit the extensive species differences found with the serum enzyme [6].

The differences in the affinities of DFP and propoxur for the two enzymes are not surprising, since the chemical structure of these inhibitors is quite different. On the other hand, the significant variation in the contribution of affinity to inhibition suggests that attempts should be made to measure the individual inhibition constants before attributing a variation in inhibition rates to differences in affinity.

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Effect of fighting and diphenylhydantoin on the uptake of ^{3}H-*l*-norepinephrine *in vitro* in synaptosomes isolated from retired male breeding mice

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In a recent study by Hendley *et al.* [1] significant increases in maximum velocity (V_{max}) and the Michaelis constant (K_m) for norepinephrine (NE) uptake in cortical homogenates were observed after the last of a series of short, intense daily fights between male mice made previously aggressive by long-term individual caging. Further observations after acute fighting showed a reversible decrease in affinity for NE uptake but no significant increase in uptake velocity [2]. The present study was likewise designed to investigate changes in central NE kinetics in acutely fighting animals, but in this instance, the effect of the drug, diphenylhydantoin (DPH, Dilantin), on NE uptake was also assessed.

The choice of DPH as a pharmacologic agent in the present study stems from earlier work reported from our laboratory in which a significant decrease in the uptake of NE in isolated rat synaptosomes incubated in physiologic media was shown after adminstration of DPH both *in vitro* [3] and *in vivo* [4]. A DPH effect on the uptake of NE in brain slices or subcellular preparations has since been confirmed by others [5, 6].

DPH was also of interest to us, since it has been observed to prevent fighting in the present animal model* as well as in electro-shocked mice [7,8]. Furthermore, there have been reports that DPH reduces aggresive behavior and anger in humans [9, 10], although studies with a contrasting conclusion have been reported [11].

In the present experiments, white male Swiss CD-1 retired breeders (Charles River Mouse Farms) were used. Since these animals often fight spontaneously,* procedures such as prolonged solitary confinement, electric shock or pharmacological manipulations are not needed to elicit aggression. The animals were divided into two separate groups upon receipt because fighting occurred more readily between strange animals than in cage-mates. After 3 days, combat pairs were obtained by taking one animal from each group and then placing them together in a confined space where they were permitted to fight vigorously. However, as with every aggressive animal model, all combat pairs did not fight equally well. Therefore, in order to obtain uniform results, it was sometimes necessary to maintain or induce fighting by applying pressure to the backs or tails of the animals with forceps. After sustained fighting for 5 min, the animals were extremely excited and there was a marked increase in respirations and heart rate. At this point, the combatants were immediately decapitated and their brains were removed within 30 sec and placed in ice-cold buffer. Pooled control brains were obtained from similar animals that were not allowed to fight.

Whole brain synaptosomes from fighting and non-fighting mice were prepared by the method of Whittaker [12]. Synaptosomal protein (0·15 to 0·3 mg per assay [13]) was preincubated in an oxygenated Krebs–Henseleit buffer for 5 min with and without 10^{-4} M DPH at 37°. An identical set of samples was incubated at 0° to serve as blanks. Then 0·05 to 2×10^{-6} M tritiated NE (New England Nuclear Corp.; spec. act., 7·2 Ci/m-mole) was added. After 5 min of additional incubation, uptake in the experimental samples was terminated by rapidly chilling the samples to 0°. After one centrifugation and washing, the pellet was solubilized (NCS-Amersham Searle), fluor (PPO/POPOP† in toluene base) was added and the samples were counted.

The uptake of ^{3}H-NE was significantly altered by fighting and by DPH in synaptosomes isolated from white Swiss male mice retired from breeding activity. The double reciprocal plot of 1/net uptake velocity in nmoles/g of synaptosomal protein/5 min) versus 1/tritiated norepinephrine, 10^{-6} M, gives the lines as shown by a linear regression least squares analysis of the data (Figs. 1 and 2). The slope and intercept were computed and, from them, V_{max} and the K_m were calculated. Each data point represents the mean value of two experiments, except for the point at 0·084 μM NE for fighting control mice. The latter data point represents a single value. The paired experiments gave values which were within a range up to 8 per cent of their mean, except for the members of one pair which were within 17 per cent of their mean.

* S. Bogoch and M. Baldwin, personal communication

† PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-2-(4-methyl 5-phenyloxazolyl) benzene.