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Disposition of aprophen in rats

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The pharmacokinetics of [^{14}C]aprophen and its distribution were determined after intravenous administration to rats. The drug was distributed rapidly with a $t_{\frac{1}{2}}$ (α) of 4 min to highly perfused organs like the brain, kidney and adrenals. An elimination phase was apparent 10 min after injection with a $t_{\frac{1}{2}}$ (β) of 23·5 min. The high plasma clearance of the drug was due both to a large volume of distribution and to a high metabolic rate. Aprophen could be hydrolysed to diphenylpropionic acid and diethylaminoethanol in-vivo and in-vitro. Diethylaminoethanol competed with [^{3}H]QNB binding to muscarinic receptors of N4TG1 cells, whereas diphenylpropionic acid did not. The lower plasma concentrations and lower binding activity of diethylaminoethanol compared with aprophen indicate that unchanged aprophen is largely responsible for the in-vivo actions.

Aprophen (2-diethylaminoethyl 2,2-diphenylpropionate, see Fig. 1) is a potent cholinolytic and antispasmodic agent (Prozorovskii 1968), which can be administered prophylactically and therapeutically as an antidote to organophosphate poisoning (Mashovskii & Roschina 1969). In a series of comparative studies in which the anticholinergic activity of aprophen and some of its structural analogues were compared with atropine, the diethylaminoethyl ester analogues showed greater ganglion-blocking activity than atropine in protecting mice poisoned with organophosphates (Mashovskii & Roschina 1969). Aprophen competes for binding to muscarinic receptors of neuroblastoma and neuroblastoma × glioma cell lines (Gordon et al 1983). We have investigated the pharmacokinetics of aprophen in the rat and the activity of a major metabolite with respect to binding to muscarinic receptors of N4TG1 cells.

Materials and methods

Materials. Aprophen was synthesized at Walter Reed according to the method of Zuagg & Horrom (1950). [^{3}H]Quinuclidinyl benzilate ([^{3}H]QNB) (33·1 Ci mmol^{-1}), d[13-^{3}H]tubocurarine (15·8 Ci mmol^{-1}), [ethyl ester-1,2-^{14}C]aprophen (353·5 mCi mmol^{-1}) and [acetyl-1-^{14}C]SCoA (46·6 mCi mol^{-1}) were purchased from New England Nuclear (Boston, MA). Diethylaminoethanol was obtained from Chemical Dynamics Corp. (South Plainfield, NJ). 2-Diethylamino ethylacetate and 2-diethylamino [1,2-^{14}C]ethylacetate (fumarate salt, 17·5 mCi mmol^{-1}) were synthesized by Research Triangle Institute (Research Triangle, NC). Carboxyesterase (EC 3.1.1.1) from porcine liver, choline acetyltransferase (EC 2.3.1.6) from bovine brain, eserine sulphate and paraoxon were purchased from Sigma Chemical Co. (St Louis, MO). Butyryl cholinesterase (EC 3.1.1.8) from human plasma, alcohol dehydrogenase (E 1.1.1.2) from horse liver, and cholesterol esterase (EC 3.1.1.13) from *Candida cylindracea* were obtained from Boehringer Mannheim.

Thin layer plates (Eastman Chromagram Silica Gel Sheet 6060) were from Eastman Kodak (Rochester, NY). AG1-X8 resin (fumarate, 200–400 mesh) was from Bio-Rad (Richmond, CA).

Pharmacokinetics of aprophen in rats. Male Sprague-Dawley rats (250–350 g) were used. A femoral artery and vein were cannulated under anaesthesia 4 days before experiments. [^{14}C]Aprophen (20 μCi) was injected in 1 ml of saline via the cannula in the vein, and was followed by 0·5 ml of heparin/saline. Blood samples (0·5 ml) were drawn from the arterial cannula 1, 5, 10, 20, 30 and 60 min after injection. Kinetic studies were performed in 5 rats. For distribution studies, the animals (n = 2) were decapitated and immediately frozen in liquid nitrogen. Organs and blood samples were homogenized in 0·067 M Na-phosphate buffer (pH 8·5), and analysed in triplicate. Aliquots (1 ml) were extracted with 10 ml of diethyl ether, evaporated to dryness and taken up in 1 ml methanol.

Unchanged aprophen was separated from the metabolites and quantified by a high pressure liquid chromatographic (HPLC) method as described by Brown et al (1980).

The pharmacokinetic data were analysed according to a two compartment open model with first order elimination kinetics. Total clearance was calculated by dose/(A/α + B/β), where A and B are y-intercepts of the extrapolated lines of the α-phase and β-phase, respectively. V_C was calculated by dividing the dose by (A + B); V_β was obtained by dividing total clearance by β.

Enzymatic hydrolysis of aprophen. A spectrophotometric method was devised to assay diethylaminoethanol formed as a result of the hydrolysis of aprophen by esterases (Fig. 1). It was based on the oxidation of diethylaminoethanol, a product of hydrolysis, by alcohol dehydrogenase in the presence of NAD (Fig. 1). The reduction of NAD to NADH was then monitored at 340 nm. The reaction mixture, in a final volume of 1 ml, consisted of 2 mM aprophen, 2 mM NAD, alcohol

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dehydrogenase (40 u) and 50 mM piperazine-*N*, *N'*-bis(2-ethanesulphonic acid) (PIPES) (pH 6·6). The reaction was carried out at 25 °C and was started by the addition of an esterase (35 u carboxyesterase; 3 u cholesterol esterase; 5 u butyrylcholinesterase).

Binding experiments. N4TG1 neuroblastoma cells were used to study [^{3}H]QNB binding as described (Gordon et al 1983), and were grown in Dulbecco's minimal essential medium, with 4·5 g glucose litre^{-1}, supplemented with 5% foetal calf serum, L-glutamine (4 mM), penicillin G (200 u litre^{-1}), and streptomycin sulphate 200 ng litre^{-1}.

I50 values were determined using the ALLFIT computer program written for an Apple computer (Munson & Rodbard 1979).

Results and discussion

After intravenous injection of [^{14}C]aprophen, the drug was distributed very rapidly to tissues. The $t_{\frac{1}{2}}$ (α) was only 3–5 min and during the distribution phase, high concentrations of radioactivity were attained in highly perfused organs like the brain, kidney and adrenals (Tables 1, 2). The elimination phase of the drug was apparent 10 min after injection as indicated by a second phase with a $t_{\frac{1}{2}}$ (β) of 20–27 min, and by an HPLC tracing of extracted plasma showing appearance of radioactivity in peaks corresponding to major metabolites. The very high systemic (total) clearance of the drug is due both to a large V_β and a high metabolic rate.

Very rapid elimination suggesting high systemic clearance was also found with adiphenine, a structural analogue of aprophen (Michelot et al 1981).

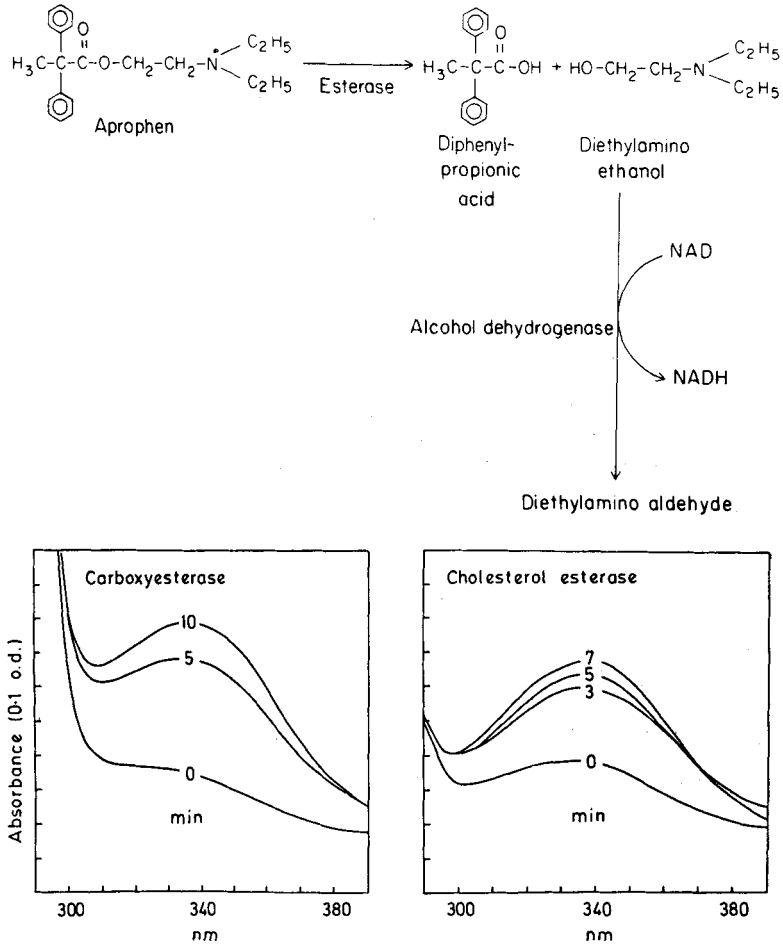

FIG. 1. Hydrolysis of aprophen by carboxyesterase and cholesterol esterase in-vitro. A spectrophotometric method was devised to assay diethylaminoethanol formed as a result of the hydrolysis of aprophen by esterases. It was based on the oxidation of diethylaminoethanol by alcohol dehydrogenase in the presence of NAD. The reduction of NAD to NADH was then monitored at 340 nM. The reaction mixture consisted of 2 mM aprophen, 2 mM NAD, alcohol dehydrogenase (40 u), and 50 mM PIPES buffer (pH 6·6). The reaction was carried out at 25 °C and was started by the addition of 35 u carboxyesterase (left) or 3 u cholesterol esterase (right).

Table 1. Pharmacokinetic data after i.v. injection of aprophen in rats.*

Kinetic variable	
$t_{\frac{1}{2}}(\alpha)$ (min)	4·0
$t_{\frac{1}{2}}(\beta)$ (min)	23·5
V_C (litre)	31·3
V_β (litre)	94·2
Cl_T (ml min^{-1})	2498·0

* Values are given as means.

Table 2. Distribution of 20 μCi [^{14}C]aprophen in rat tissues after i.v. injection.*

	5 min	3 hrs
Organ	d min^{-1} g^{-1}	
Adrenals	49 365	17 400
Lungs	31 328	5 125
Kidney	27 359	3 201
Spleen	24 552	6 331
Small intestine	21 578	13 909
Heart	16 699	3 860
Brain	10 265	1 381
Liver	8 354	4 361
Muscle	6 560	1 517

* Values are given as means.

HPLC tracings of rat plasma showed several radioactive peaks apart from the aprophen peak appearing 5 min after i.v. injection of [^{14}C]aprophen (not shown). The hydrolytic product diethylaminoethanol appeared 30 min after injection, but was not detectable after 120 min. The hydrolytic cleavage of aprophen to diphenylpropionic acid and diethylaminoethanol was carried out by carboxyesterase and by cholesterol esterase (Fig. 1). However, human plasma butyrylcholinesterase up to 5 u was incapable of hydrolysing aprophen. The major site of aprophen metabolism in-vivo is unknown, but the liver is a likely site for formation of the numerous metabolites detected in rat urine after its administration (unpublished).

The cholinolytic actions of aprophen in-vivo could be due to the action of the drug itself or to active metabolites. To test the activity of the hydrolytic products, their ability to block the binding of [^{3}H]QNB

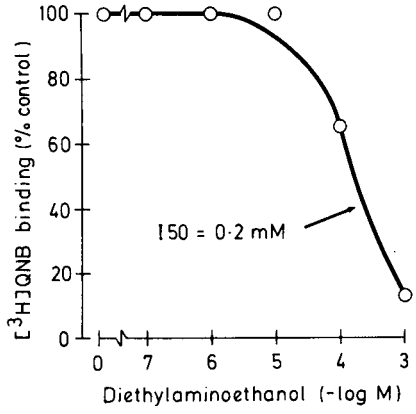

FIG. 2. Inhibition of [^{3}H]QNB binding by diethylaminoethanol to the muscarinic receptor of N4TG1 neuroblastoma cells.

to the muscarinic receptors of N4TG1 cells was examined. Diphenylpropionic acid did not compete with QNB binding. Diethylaminoethanol competed with QNB for muscarinic binding sites (Fig. 2) with a K_i of 4×10^{-4} M. Since plasma levels of the metabolite were lower than that of the parent compound for the first 60 min after injection and the K_i of aprophen was 100 times lower (4×10^{-6} M) than that of the metabolite, the data indicate very strongly that diethylaminoethanol contributes very little to the in-vivo effects compared with unchanged aprophen.

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