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Phthalic acid esters inhibit arachidonate metabolism by rat peritoneal leucocytes

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Phthalic acid esters concentration-dependently inhibited the formation of both cyclo-oxygenase and lipoxygenase arachidonate products by rat peritoneal leucocytes. Phthalates are extracted by human transfusion blood stored in pvc bags, and might similarly affect the blood cells when administered to patients.

Phthalic acid esters are used extensively as plasticisers and form 30 to 40% of various plastics. These can enter human tissues (Thomas & Northup 1982) and although they have low acute toxicity the consequences of chronic intake have not been fully determined (Rock et al 1978).

We have found that phthalic acid esters can affect the tone of rat gastric fundus and its responses to PGE_2 and acetylcholine (Tavares et al in press). We now report the effects of diethylhexyl phthalate (DEHP), its metabolite monoethylhexyl phthalate (MEHP), and dibutyl phthalate (DBP) on arachidonic acid metabolism by rat peritoneal leucocytes.

Methods

Rat resident peritoneal leucocytes, obtained by washing the peritoneal cavity with Dulbecco's phosphate buffered saline (pbs), were suspended in pbs (700 000 cells ml^{-1}). Aliquots of cells (0·7 ml) were pre-incubated (37 °C, 15 min) with pbs alone (0·2 ml) or containing 1, 10 or 100 μg of a phthalate. This was followed by a further incubation for 5 min at 37 °C with [1-^{14}C]-arachidonic acid (0·1 μCi, 1·7 nM) and calcium ionophore A23187 (1 μg) to give a final volume of 1 ml. Enzyme activity was terminated with methanol: formic acid (1 ml : 10 μl), the eicosanoids were extracted with diethyl ether (3 ml × 2) and the dried extracts chromatographed on silica gel thin layer plates (Eastman Kodak) using hexane–ether–acetic acid (40 : 60 : 3) together with standards of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), 5-hydroxy-eicosatetraenoic acid (5-HETE) and arachidonic acid. Autoradiographs were prepared, the TLC tracks were cut into segments, and the radioactivity was determined by scintillation counting.

Results

Rat peritoneal leucocytes stimulated by calcium ionophore A23187 metabolised [1-^{14}C]arachidonic acid via both the cyclo-oxygenase and liposygenase pathway (Siegel et al 1981). By comparison with authentic standards the products were determined as prostaglandins (measured collectively), LTB_4, 5-HETE, 11-HETE and 15-HETE. The control mean percent incorporations of [^{14}C] were 7·2, 1·3, 1·3, 3·0 and 3·3% respectively.

The phthalic acid esters concentration-relatedly inhibited the formation of PGs, LTB_4, 5-HETE and 15-HETE by rat peritoneal leucocytes (Table 1). The order of potency was DBP>MEHP>DEHP. Whereas DEHP and MEHP also concentration-relatedly inhibited 11-HETE formation, DBP caused an inhibition only at 100 μg ml^{-1}.

Discussion

Phthalic acid esters, DBP in particular, have some structural resemblance to prostaglandins, and this may explain why the phthalates inhibited cyclo-oxygenase and lipoxygenase activity by rat peritoneal leucocytes.

Table 1. The effect of phthalic acid esters on arachidonic acid metabolism by rat peritoneal leucocytes. Results are expressed as the mean percentage of controls ± se. n = 6. Statistical significance was determined by the Student's paired *t*-test. *P* values: a<0·02, b<0·005, c<0·001.

DEHP	PGs	LTB_4	5-HETE	11-HETE	15-HETE
1 μg ml^{-1}	93·2 ± 9·3	87·3 ± 5·8	80·3 ± 4·9	84·7 ± 3·0[b]	92·5 ± 11·6
10 μg ml^{-1}	83·6 ± 7·9	75·7 ± 4·0[b]	69·9 ± 2·6[c]	70·8 ± 3·5[c]	82·0 ± 9·3
100 μg ml^{-1}	64·3 ± 4·8[b]	70·3 ± 4·1[c]	61·4 ± 2·9[b]	63·0 ± 3·8[c]	64·2 ± 2·5[c]
MEHP					
1 μg ml^{-1}	97·9 ± 6·9	109·8 ± 5·0	96·0 ± 8·0	82·1 ± 13·0	102·2 ± 5·4
10 μg ml^{-1}	81·9 ± 14·0	92·5 ± 11·7	82·9 ± 15·4	60·6 ± 8·5[a]	79·4 ± 14·8
100 μg ml^{-1}	39·9 ± 11·9[a]	53·5 ± 13·9[a]	44·5 ± 9·9[b]	29·7 ± 5·3[c]	36·1 ± 5·7[c]
DBP					
1 μg ml^{-1}	79·1 ± 15·4	89·7 ± 8·5	77·8 ± 4·5[a]	106·4 ± 16·7	86·8 ± 11·4
10 μg ml^{-1}	42·5 ± 9·0[b]	88·2 ± 15·6	80·3 ± 14·1	128·4 ± 26·7	55·6 ± 12·0[a]
100 μg ml^{-1}	23·2 ± 3·7[c]	53·9 ± 6·4[c]	44·3 ± 5·8[c]	46·0 ± 5·7[b]	28·1 ± 3·1[c]

The inhibition of dog platelet aggregation by phthalates (Rubin & Jaeger 1973) might therefore be due to inhibition of thromboxane synthesis.

DEHP is the most widely used plasticizer and is therefore to be found almost everywhere in the western world. Transfusion blood stored in polyvinyl chloride plastic bags can extract DEHP at a daily rate of 2·5 μg ml^{-1} (Jaeger & Rubin 1972) which is then metabolized to MEHP by plasma proteins (Rock et al 1978), and up to 300 μg ml^{-1} have been found in plasma products (Cole et al 1981).

Our results raise the possibility that phthalic acid esters infused into patients might inhibit arachidonate metabolism, and alter cell functions such as platelet aggregation. It is also possible that phthalates could form the basis of anti-inflammatory drugs that inhibit both cyclo-oxygenase and lipoxygenase activity.

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Comparative evaluation of the effects of lidocaine (lignocaine) hydrochloride and salicylate on nervous and purkinje fibres

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Lidocaine hydrochloride and lidocaine salicylate (lisacaine) have been tested on the frog node of Ranvier, on the sheep cardiac Purkinje fibres and rat phrenic nerve-diaphragm. On the node of Ranvier both drugs produced the same degree of reduction of peak I_{Na} and steady-state potassium current and the same degree of shift of the steady-state inactivation curve for I_{Na} to more negative potential. Lisacaine took less time to reach the steady-state effect. On the cardiac Purkinje fibres both drugs decreased the action potential duration without any detectable difference; but their effects on V max (i.e. the maximum rate of depolarization) were different, that of lisacaine being faster. On the rat phrenic nerve-diaphragm both drugs produced the same percentage of reduction of the contractile response of diaphragm but, the action of lisacaine was faster. Therefore the lidocaine molecule with the salicylate anion while displaying the same anaesthetic effectiveness has a faster action than the hydrochloride.

The effects of lidocaine (lignocaine) have been widely investigated on myelinated nerve fibres and have been explained in terms of local anaesthetic receptor interaction (Århem & Frankenhaeuser 1974; Hille 1977; Schmidtmayer & Ulbricht 1980). The salicylate ions, used clinically to produce anti-inflammatory, analgesic and antipyrexic effects, were investigated on the frog node of Ranvier (Attwell et al 1979), squid giant axon (Neto & Narahashi 1976), and sheep cardiac Purkinje fibres (Cohen et al 1979). On the node of Ranvier and squid giant axon the salicylate compounds were found to reduce maximum sodium and steady-state potassium conductances. Attwell et al (1979), to explain the effects on the node of Ranvier, envisaged that salicylate ions are incorporated into the outer surface of the membrane thereby changing the surface potential. Cohen et al (1979) also explain the prolongation of action potential duration and the increase of resting potential produced by salicylate ions on sheep cardiac Purkinje fibres in terms of surface potential changes. Taking into account these properties of salicylate ions, we have evaluated the effects of lidocaine hydrochloride and salicylate comparatively in the frog node of Ranvier, sheep cardiac Purkinje fibres and rat phrenic nerve-diaphragm preparations, to establish whether the salicylate ions can modify the anaesthetic properties of the lidocaine molecule.

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Methods

Myelinated nerve fibres were isolated from the sciatic nerve of the frog, *Rana esculenta*. The action potential and membrane currents were recorded under current clamp and voltage clamp conditions using the method of Nonner (1969). The resting potential was assumed to be −70 mV, corresponding to a 30% fast Na inactivation. The current amplitude was calculated assuming an axoplasmic resistance of 10 M ohms. Under voltage clamp conditions, the Na resting inactivation was removed by 50 ms hyperpolarizing prepulses to −160 mV. The normal Ringer solution had the following composition (mM): NaCl, 111·5; KCl, 2·5; $CaCl_2$, 1·8; CO_3HNa, 2·4. The two cut ends of the fibre were bathed in an isotonic KCl (117 mM) solution plus 5 mM Hepes (Sigma) (pH 7). In most of the cases, Na currents