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

were recorded after blockade of the currents through K channels by external tetraethylammonium (10 mM) and replacement of the internal KCl by 80 mM CsCl (Dubois & Bergman 1975). The temperature was 14 ± 0·5 °C.

Intracellular action potentials were recorded from isolated sheep cardiac Purkinje fibres. Details of the procedures are given elsewhere (Mugelli et al 1983). In brief, the preparations were mounted in a tissue bath (0·3 ml) and superfused with oxygenated (97% O_2, 3% CO_2) warm (37 °C) Tyrode solution and driven at constant rate, as indicated in the text. The transmembrane potentials were recorded by means of two microelectrodes filled with 3 M KCl. The maximum rate of depolarization (V max) was measured by recording the first derivative obtained by electronic differentiation (range $1–10^5$ V/s) (Label).

The method described by Bulbring (1946) was used for the experiments on the rat phrenic nerve-diaphragm preparation which was mounted in a 40 ml organ bath and superfused with oxygenated (95% O_2, 5% CO_2) warm (37 °C) Ringer solution of the following composition (g $litre^{-1}$): NaCl 8; KCl 0·4; $CaCl_2$ 0·3; $MgSO_4$ H_2O 0·2; NaH_2PO_4 H_2O 0·2; Na_2CO_3 0·1; glucose 1. The diaphragm twitch response induced by phrenic nerve stimulation with supramaximal shocks delivered from an electronic square-wave stimulator (0·1 V, 4 min^{-1}, 0·1 ms) were recorded isometrically with a DYO microdinamometer (Basile) under a tension of 10 mg.

The test drugs, lidocaine hydrochloride and lidocaine salicylate (lisacaine) were provided by Molteni (Florence, Italy). All doses in the text refer to the concentration in the bathing medium.

Results and discussion

After addition of 0·25 mM lidocaine or lisacaine to the external solution superfusing the node of Ranvier, current clamp and voltage clamp mesurements were made. The action potential was almost abolished without any change in the resting potential (Fig. 1). The results of voltage clamp experiments are presented in Table 1: the peak sodium and steady-state potassium currents, recorded during depolarizations of various amplitudes, were respectively reduced by 60 and 20% without any detectable difference between the two drugs. Lidocaine and lisacaine shifted the steady-state sodium inactivation-voltage curve (h∞) towards negative voltage by about 15 mV at 50% inactivation and decreased the slope of the curve. The effects of

Table 1. Voltage clamp measurements from the node of Ranvier in the presence of lidocaine or lisacaine.

	I_{Na} (% decrease)	I_K (% decrease)	*h∞ (negative shift)	**Kd
Lidocaine (0·25 mM)	60	20	15 mV	Lidocaine 0·2 mM
Lisacaine (0·25 mM)	60	20	15 mV	Lisacaine 0·2 mM

* h∞ was evaluated recording the peak sodium current during test pulses to 0 mV preceded by 50 ms pulses of various amplitudes. The shift was measured at 50% inactivation.

** Kd: dissociation constant calculated from the dose-response curve of lidocaine and lisacaine on peak sodium current recorded during pulses to 0 mV. A one to one reaction between channels and drug molecules is assumed.

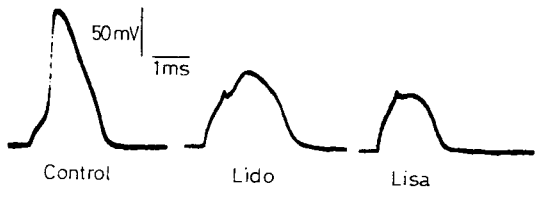

FIG. 1. Action potential recorded from the node of Ranvier in control Ringer solution and in the presence of 0·25 mM lidocaine (Lido) or lisacaine (Lisa). Pulse duration 0·1 ms, frequency <0·2 Hz.

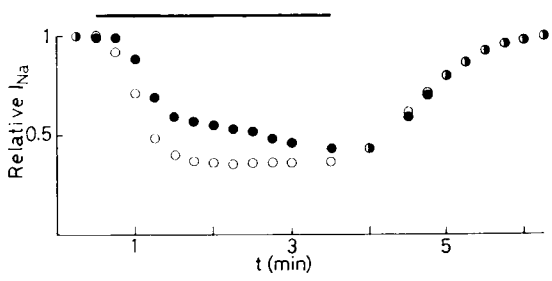

FIG. 2. Kinetics of effects on peak Na current recorded from the node of Ranvier. The current, represented normalized to its value in control Ringer solution, was recorded during pulses to 0 mV every 15 or 30 s before, during (horizontal bar) the application of 0·25 mM Lido (●) or Lisa (○) and during wash out.

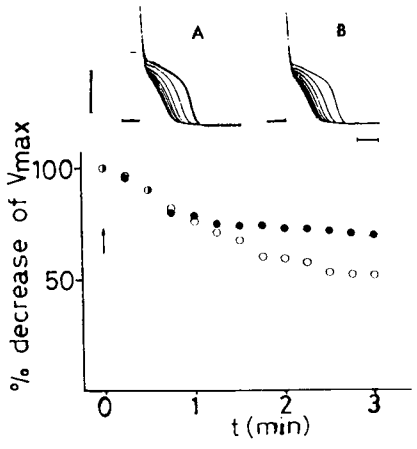

FIG. 3. Effects on Purkinje fibres action potential and V max. Upper panels: superimposed action potentials recorded every 15 s in the absence (external trace) or in the presence of 0·25 mM Lido (A) or Lisa (B). Calibrations: vertical 50 mV, horizontal 100 ms. Lower panel: V max was recorded in control and during the application of 0·25 mM Lido (●) or Lisa (○). The preparations were paced at 4 min^{-1}.

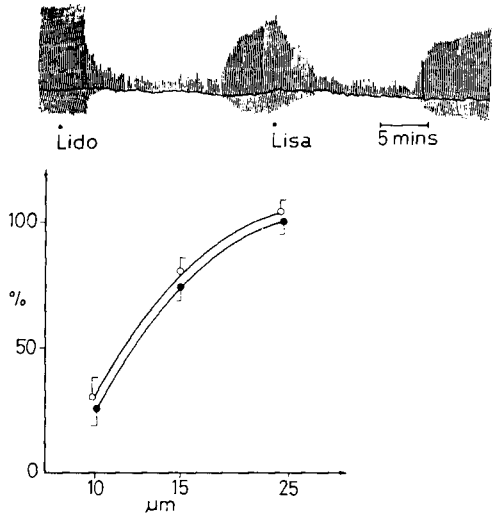

FIG. 4. Effects on phrenic nerve—diaphragm prepartion. Upper panel: twitch response of diaphragm induced by phrenic nerve stimulation with rectangular pulses of 0·1 mV under 0·15 μM Lido or Lisa. Lower panel: dose-response curve of Lido (●) or Lisa (○). Abscissa scale: drugs concentration plotted on a logarithmic scale; ordinate: percentage of reduction of diaphragm twitch response.

increasing concentrations of lidocaine and lisacaine were tested on relative peak Na current recorded during pulses to 0 mV: between 50 μM and 1 mM the effects of both drugs were similar. The block can be described by a one to one reaction between the channels and the drug molecules with an apparent dissociation constant of 0·2 mM (see Table 1). Fig. 2 presents the kinetics of effects on peak sodium current and recovery from block. This kind of analysis shows a difference between the two drugs since I_{Na} block by lisacaine was faster than I_{Na} block by lidocaine.

The effects of the two drugs on action potential duration and on maximum rate of the depolarization (V̇ max), were studied in cardiac Purkinje fibres driven at 4 min^{-1} to allow a beat-to-beat analysis of the effect development. The action potential duration was decreased by both drugs without any detectable difference and the maximal effect was reached after 1·30 min; however, as shown in Fig. 3, the effects of lidocain and lisacaine on V̇ max were different; Lisacaine caused a faster and greater reduction of V̇ max than lidocaine. Dose-response curves of lidocaine and lisacine on the phrenic nerve-diaphragm preparation are shown in Fig. 4. Both drugs decreased the diaphragm twitch response elicited by the phrenic nerve stimulation without any detectable difference; however lisacaine produced a faster reduction of the contractile response as shown in the mechanogram of the Fig. 4.

The results obtained in the frog node of Ranvier, sheep Purkinje fibres and rat diaphragm preparation indicate that lidocaine and lisacaine produce similar effects. So it could be deduced that by salifying the lidocaine molecule with salicylate ions the anaesthetic effectiveness is not substantially modified. It is noteworthy that in each preparation a constant difference was found between lidocaine and lisacaine with regard to the time course of development of their effects, the action of lisacaine being found to be the faster in each preparation. Therefore, the presence of the salicylate ions does not substantially modify the effectiveness of the lidocaine molecule; this was to be expected by considering that the concentrations of salicylate in the lidocaine molecule are undoubtedly lower than the concentrations of salicylate alone found effective on nerve fibres by Attwell et al (1979) and on the atrial muscle of the rabbit by Neto (1982). On the other hand, the results obtained from the analysis of the kinetics of effects of both drugs has shown that the action of lisacaine is faster; therefore, we could hypothesize that salicylate ions (at least at the concentrations used to salify the lidocaine moelcule) which are incorporated into the outer surface of the membrane, should increase the surface negative potential slightly (Attwell et al 1979; Cohen et al 1979), in so doing they could cause the lidocaine molecule to penetrate more easily the membrane and reach its site of action which is supposed on the internal surface of the membrane.

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