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Anti-inflammatory effects of indomethacin ester incorporated in a lipid microsphere

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A lipid microsphere (Intralipid), with particle size of 0·2 μm and used for parenteral nutrition in man, is taken up readily by some inflammatory cells (Hallberg 1965). The microsphere is much more stable than liposomes. The anti-inflammatory activity of dexamethasone incorporated into the lipid microsphere (liposteroid) was described by (Mizushima et al 1982a, b). We have now incorporated an indomethacin ester, indomethacin ethoxycarbonylmethyl ester, into the lipid microsphere (lipo-indomethacin) and compared its anti-inflammatory activity with that of sodium indomethacin in rats.

Method

Indomethacin ethoxycarbonylmethyl ester was synthesized as follows. A solution of ethylbromoacetate (1·7 g, 0·01 mol) in 5 ml of benzene was added to a solution of indomethacin (3·6 g, 0·01 mol) and 1,8-diazabicyclo [5,4,0]-7-undecene (1·5 g, 0·01 mol) in 50 ml of benzene, and the mixture was stirred at room temperature (20 °C) for 2 h. The reaction mixture was then washed with water, dried over anhydrous magnesium sulphate, and evaporated in a vacuum. Recrystallization from ethylacetate-n-hexene gave 2·4 g (54%) of the ethoxycarbonylmethylester of indomethacin as pale yellow needles: m.p. 82–84 °C. This ester was identified physicochemically by n.m.r. and i.r. spectra. Most of indomethacin ethoxycarbonylmethyl ester is probably converted in-vivo to indomethacin in the rats, since indomethacin carboxymethyl ester (acemetacin) with a similar chemical structure, was found to be metabolized almost completely into indomethacin in the rats (Kabuto et al 1981). In a preliminary experiment (unpublished), most of ethoxycarbonylmethyl ester was converted to indomethacin, when lipo-indomethacin was incubated with an homogenate of rat liver, and ethoxycarbonylmethyl ester itself was not anti-inflammatory like acemetacin (Mizushima 1982) in a local carrageenan paw oedema test.

Indomethacin ethoxycarbonylmethylester was dissolved in soybean oil at 124 mg ml^{-1}, and lipo-indomethacin containing 1·24 mg ml^{-1} of the indomethacin ester was prepared in a manner similar to that described by Mizushima et al (1982a, b). Lipo indomethacin thus prepared was very stable even at room temperature (20 °C). Lipo-indomethacin and sodium indomethacin solution were appropriately diluted with 0·9% NaCl (saline) before use.

Male rats of Wistar strain, 200–250 g, were used. A dose of 0·05 ml of 1% λ-carrageenan was injected into the hind paw. Two h after the carrageenan injection, the animals were divided randomly into several groups of 7 rats for each treatment, and were injected with lipo-indomethacin, indomethacin or saline intravenously. The paw volume was measured before and after the carrageenan injection. A statistical analysis was made on the inhibitory activity on carrageenan oedema

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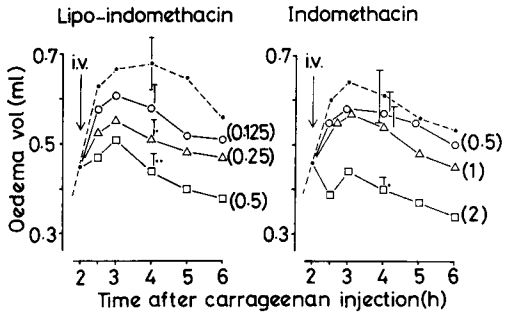

FIG. 1. Inhibitory effects of lipo-indomethacin (indomethacin ethoxycarbonylmethyl ester incorporated in a lipid microsphere) and sodium indomethacin on carrageenan paw oedema in rats. The two indomethacin preparations were injected intravenously 2 h after the carrageenan injection. Each point refers to an average value of 7 animals. I, T are s.e. at 4 h. *$P<0·05$, **$P<0·01$ in relation to the control groups. ●– –● control, numbers in parentheses show drug dose in mg equiv kg^{-1}.

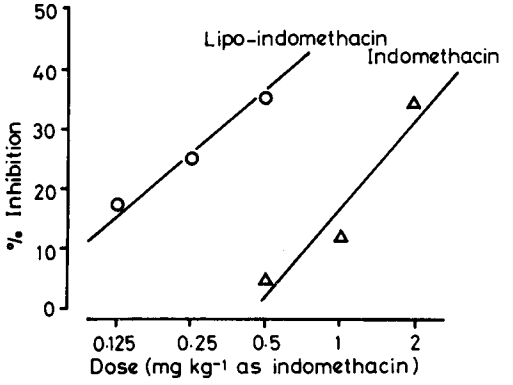

FIG. 2. Dose response curve of inhibitory effects of lipo-indomethacin and sodium indomethacin on carrageenan paw oedema in rats. The two indomethacin preparations were injected intravenously 2 h after carrageenan injection and the volume of rat paw oedema was measured 2 h later.

of the indomethacin preparation 2 h after its administration.

Results

As shown in Fig. 1, lipo-indomethacin at 0·25 and 0·5 mg kg^{-1} and sodium indomethacin at 2 mg kg^{-1} inhibited carrageenan oedema significantly. The lipid microsphere alone did not inhibit the oedema.

The dose-response curves of inhibitory effects at 4 h of lipo-indomethacin and sodium indomethacin on carrageenan oedema are shown in Fig. 2. The anti-inflammatory activity of lipo-indomethacin seemed to be about 5 times as potent as that of free indomethacin, at the 30 percent inhibitory dose. As in an earlier study using carrageenan oedema, the lipid particles (liposteroid) were highly distributed to the inflamed paw (Mizushima et al 1982b). These results suggest that lipo-indomethacin is also taken up by some inflammatory cells in carrageenan oedema more selectively than free indomethacin, resulting in a much stronger anti-inflammatory activity.

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Brain TRH receptors are the same as pituitary TRH receptors

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Thyrotropin releasing hormone (L-pyroglutamyl-L-histidyl-L-prolineamide, Pyr-His-Pro NH_2, TRH) is known to release both thyroid stimulating hormone (TSH) and prolactin. In addition the tripeptide is known to be widely distributed in the c.n.s. and to exert a variety of neuropharmacological effects. As a consequence there has been speculation that TRH may exert a role in brain function as a neuromodulator or endogenous ergotrophic substance (see Metcalf & Dettmar 1981). Injected TRH has a very short biological half-life (Bassiri & Utiger 1973) and various analogues of the tripeptide have been synthesized with the aim of increasing resistance to metabolism so that the clinical potential for the neuropharmacological actions attributed to TRH can be explored. Some of these analogues have been claimed to be more 'specific' for the c.n.s. because they exhibit enhanced potency compared with TRH in neuropharmacological screening tests (see Metcalf 1982). Such relative specificity would be possible if TRH 'receptors' in the brain differed from those in the pituitary gland. However Burt & Taylor (1980) using binding techniques have concluded that the two sites are very similar. RX 77368 (L-pyroglutamyl-L-histidyl-L-3,3-dimethylprolineamide, Pyr-His-L-Dmp.NH_2), is an analogue of TRH with pronounced potency in neuropharmacological screening tests (Metcalf et al 1982) and increased resistance to metabolic breakdown (Brewster et al 1981). To explore further whether it is possible to differentiate between TRH 'receptors' in the c.n.s. and the pituitary we have compared RX 77368 to its D-stereoisomer RX 77369 (L-pyroglutamyl-L-histidyl-D-3,3-dimethylprolineamide, Pyr-His-D-Dmp.NH_2) with respect to their ability to induce the release of TSH in rats in-vivo and their ability to reverse the hypothermia induced by reserpine in mice.

The methods used have been described before (Brewster et al 1980). Briefly, male mice (18–22 g) were used for the reserpine reversal test. They were dosed with reserpine (2 mg kg^{-1} s.c.) and 17 h later divided into groups of 8 before further treatment. The oesophageal temperature of each animal was recorded before and at intervals of 0·5, 1, 2 and 4 h following intravenous drug administration. The mean area under the 4 h temperature rise/time curve was calculated for each dose level and used to prepare log dose/response lines. Male rats (160–180 g) were used to measure TSH release. Twenty minutes after intravenous dosage blood samples were obtained by cardiac puncture and plasma separated by centrifugation (750 g, 20 min, at 4 °C). Plasma samples were stored at −20 °C until assayed. The levels of TSH in the samples of plasma were measured by a double-antibody rat radio-immunoassay according to the recommendations supplied with the kits kindly donated by the NIAMDD (NIH, USA) rat hormone distribution programme. Plasma samples were assayed against the NIAMDD-Rat TSH-RP-1 reference standard and the hormone levels expressed as ng TSH ml^{-1} of plasma.

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