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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REFERENCES

Hallberg, D. (1965) Acta Physiol. Scand. 65: suppl 254

Kabuto, S., Yonemitsu, M., Matsumoto, O., Koide, T., Nakao, H., Ishihama, H., Suzuki, J. (1981) Jpn. Pharmacol. Ther. 9: 4961–4975

Mizushima, Y. (1982) Pharmacology 25 (Suppl. 1): 39–45

Mizushima, Y., Hamano, T., Yokoyama, K. (1982a) J. Pharm. Pharmacol. 34: 49–50

Mizushima, Y. Hamano, T., Yokoyama, K. (1982b) Ann. Rheum. Dis. 41:263-267

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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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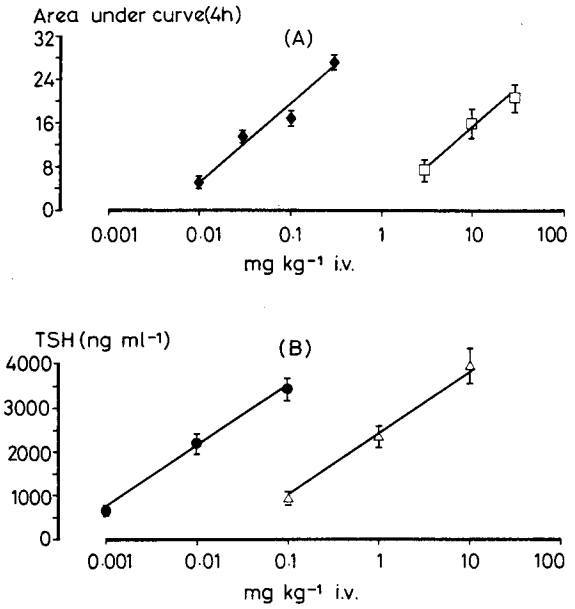

FIG. 1. A. The ability of Pyr-His-L-Dmp.NH_2 (◆) and Pyr-His-D-Dmp.NH_2(□) to reverse the hypothermia induced in mice by reserpine (2 mg kg^{-1} s.c.). Each value represents the mean (± s.e.m.) area under the 4 h temperature rise/time curve of a group of 8 mice. All animals were dosed by the intravenous route. B. The effect of Pyr-His-L-Dmp.NH_2 (●) and Pyr.His-D-Dmp.NH_2 (△) on plasma levels of TSH at the time of peak effect (20 min) after intravenous administration to rats. Each value represents the mean (± s.e.m.) response where n = 8.

The results obtained are illustrated in Fig. 1. It can be seen that both Pyr-His-L-Dmp.NH_2 and Pyr-His-D-Dmp.NH_2 caused a dose-related reversal of reserpine-induced hypothermia in the mouse. In terms of relative potency the D-isomer had approximately 0·01 the potency of the L-isomer. As would be predicted, the DL-racemate Pyr-His-DL-Dmp.NH_2 was intermediate between the two isomers. Similarly in terms of TSH release both Pyr-His-L-Dmp.NH_2 and Pyr-His-D-Dmp.NH_2 caused a dose related release of the pituitary hormone and the D-isomer was about 0·02 times as potent as the L-isomer.

In a parallel series of experiments we compared the potency of TRH with its 3-methyl-histidine analogue (Pyr-3MeHis-ProNH_2) and obtained similar results. In the TSH release experiments the analogue was eight times more potent than TRH whilst it was five times more potent than the parent peptide in the reserpine reversal test.

Thus the present results demonstrate that D-and-L-isomers of the same structure (i.e. Pyr-His-L-Dmp.NH_2 and Pyr-His-D-Dmp.NH_2) or closely related structures (i.e. Pyr-His-ProNH_2 and Pyr-3MeHis-ProNH_2) exhibit similar relative potencies in either c.n.s. or endocrine tests. This result is consistent with the conclusion that the 'receptors' involved at both sites are very similar if not identical. Such a conclusion agrees with that reached by Burt & Taylor (1980) using receptor binding techniques. However the fact remains that several analogues of TRH, including RX 77368, have attracted attention because the results from animal testing suggest that the analogues exhibit enhanced c.n.s. selectivity. Previously we have argued that such apparent selectivity does, in fact, result from the metabolic stability of the analogues so more of the administered dose is available to cross the blood brain barrier and exert its c.n.s. effects (Metcalf et al 1981) rather than differential effects at different receptors. Further enhancement of the central activity exerted by TRH analogues would result from improvements in their ability to cross the blood brain barrier.

REFERENCES

Bassiri, R. M. Utiger, R. D. (1973) J. Clin. Invest. 52: 1616–1619

Brewster, D., Dettmar, P. W., Lynn, A. G., Metcalf, G., Morgan, B. A., Rance, M. J. (1980) Eur. J. Pharmacol. 66: 65–71

Brewster, D., Humphrey, M. J., Wareing, M. V. (1981) Neuropeptides 1: 153–165

Burt, D. R., Taylor, R. L. (1980) Endocrinology 106: 1416–1423

Metcalf, G. (1982) in: Bennett, G. W., Griffiths, E. C. (eds) Proceedings of the ISN Satellite Symposium on TRH. Raven Press. New York (in the press)

Metcalf, G., Dettmar, P. W. (1981) Lancet 1: 586–589

Metcalf, G., Dettmar, P. W., Fortune, D., Lynn, A. G., Tulloch, I. F. (1982) Reg. Peptides 3: 193–206

Metcalf, G., Dettmar, P. W., Lynn, A. G., Brewster, D., Havler, M. E. (1981) Ibid. 2: 277–284