Table 2. Effects upon cAMP phosphodiesterase (PDE) activity.

Drug	Organ	IC50 (with confidence limits) μM (as theophylline)
Reproterol	Lung	705 (413–1190)
	Heart	444 (261–756)
	Aorta	1000 (534–1890)
	Intestine	1310 (693–2510)
Theophylline	Lung	779 (459–1310)
	Heart	469 (261–833)
	Aorta	1030 (582–1800)
	Intestine	1319 (731–2400)

provides evidence that, for reproterol, the inhibition of 3'5'-monophosphate phosphodiesterase nucleoside (PDE), which hydrolyses cAMP to AMP and which controls the intracellular concentration of cAMP (Robinson et al 1974), is one of the molecular mechanisms underlying its anti-asthma effects. This effect can be attributed to the presence of theophylline in the reproterol molecule and is additional to its ability to activate the β-adrenoceptors directly. The release of inflammatory mediators (histamine, SRS-A, etc) is inhibited by the intracellular rise in cAMP (Bourne et al 1974) through a mechanism which is not known but which involves the reduction of the permeability of mast cells to calcium (Foreman et al 1975).

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Stabilization of a sustained release-type injection vehicle for a synthetic corticotrophin analogue

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Adrenocorticotrophin (ACTH) is a pituitary peptide hormone which stimulates the adrenal cortex to secrete corticosteroids. Preparations of ACTH and its synthetic analogues are currently used to test adrenal functions or to clinically treat rheumatoid arthritis and asthma (Haynes & Murad 1980). Since the biological half-life of ACTH is short, various depot-type injections have been devised for therapeutic treatment using gelatin, zinc hydroxide or carboxymethyl cellulose (British Pharmacopoeia 1980; Hedner 1963). Adsorption of the peptide onto the zinc hydroxide (or zinc phosphate) dispersion in the vehicle results in marked prolongation of corticotrophic activity of ACTH upon intramuscular injection, which could be due to either delayed absorption into circulating blood or protection of the peptide from enzymic inactivation at the extravascular injection site. The formulation can be applied to synthetic short-chain ACTH analogues such as [Gly^1]-ACTH-(1-18)-NH_2 (Otsuka & Inouye 1975; Tanaka 1971). Although freshly prepared zinc-[Gly^1]-ACTH-(1-18)-NH_2 dispersion showed a pronounced depot effect when injected into rat thigh muscle, the dispersion became aggregated within 24 h when stored at 25 °C and the depot effect was lost. However, the dispersion could be stabilized by including histidylhistidine (Hirata et al 1974) making possible a long-lasting corticotrophic activity.

The zinc-[Gly^1]-ACTH-(1-18)-NH_2 dispersion was prepared by first mixing 10 mg [Gly^1]-ACTH-(1-18)-NH_2, 5 mg L-histidyl-L-histidine, 90 mg NaCl, 100 mg benzyl

* Correspondence.

alcohol, 2 ml of 100 mM $ZnCl_2$, 1·4 ml of 100 mM Na_2HPO_4 (pH 3 with HCl) and 4·9 ml of distilled water, then adding 1·6 ml of 0·2 M NaOH to adjust the pH to 7·0. Portions of 10 µl of the resulting suspension were injected into the thigh muscle of male Wistar rats (110 to 130 g), 4 h after hypophysectomy. Blood samples were collected at intervals from the abdominal aorta (each datum value represents four rats). The plasma concentration of 11-hydroxycorticosterone (11-OHCS) was determined by fluorimetry (Hirata et al 1981). As a control, 10 mg [Gly¹]-ACTH-(1-18)-NH_2 was dissolved in 10 ml phosphate buffer (20 mM Na_2HPO_4, pH 7·0, with 0·9% NaCl and 1% benzyl alcohol), and 10 µl of this clear solution was injected intramuscularly.

Table 1. Corticotrophic activity of zinc-[Gly¹]-ACTH-(1-18)-NH_2 dispersion after storage at various temperatures. Corticotrophic activities of stored samples are expressed as plasma 11-OHCS 4 and 5 h after injection of the preparations to hypophysectomized rats. (n = 4). Means ± s.e.

Storage conditions	Plasma 11-OHCS (µg/100 ml) 4 h	5 h
—	29·1 ± 3·5	33·4 ± 4·0
4 °C, 16 months	29·1 ± 0·8	34·1 ± 3·0
37 °C, 12 months	26·5 ± 3·5	28·7 ± 2·0
45 °C, 3 months	32·1 ± 1·4	30·5 ± 5·9
45 °C, 6 months	29·0 ± 2·0	27·6 ± 1·1

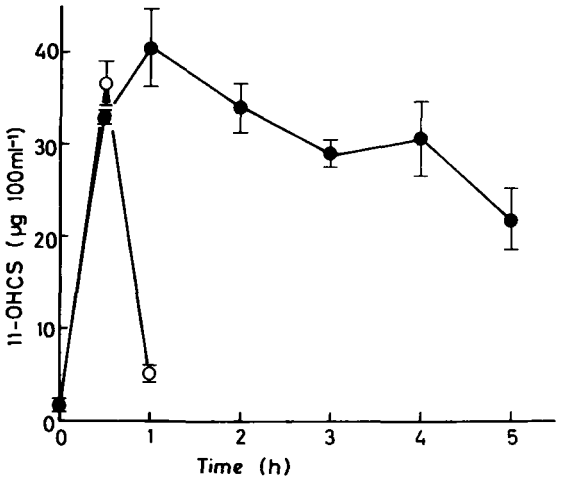

FIG. 1. Time course of plasma 11-OHCS level in hypophysectomized rats following intramuscular injection of [Gly¹]-ACTH-(1-18)-NH_2 (○—○) and zinc-[Gly¹]-ACTH-(1-18)-NH_2 (●—●). Means ± s.e.

As shown in Fig. 1, zinc-[Gly¹]-ACTH-(1-18)-NH_2 dispersion exerted a depot corticotrophic effect persisting over 5 h, whereas the effect of [Gly¹]-ACTH-(1-18)-NH_2 diminished soon after the injection.

To test the stability of zinc-[Gly¹]-ACTH-(1-18)-NH_2, the dispersion was stored in vials for several months at 4, 37 and 45 °C, and the corticotrophic activity in 10 µl samples was determined using as an index of depot effect plasma 11-OHCS levels 4 and 5 h after injection of the preparations. Based on appearance and corticotrophic activity, the preparations were judged to be stable and effective after a year at room temperature (20 °C) (Table 1).

Since histidylhistidine has neither corticotrophic activity nor a stimulating effect on the activity of ACTH, the dipeptide apparently stablizes the suspension. Zinc phosphate dispersion stabilized by histidylhistidine may serve as a useful vehicle for prolonging the activities of other peptide hormones.

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