Br. J. Pharmacol. (1992), **106**, 489–492

Peripheral analgesic activities of peptides related to α-melanocyte stimulating hormone and interleukin-1β[193–195]

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1 The hyperalgesic effects of interleukin-1β (IL-1β) and prostaglandin E_2 (PGE_2) were measured in rats.

2 Hyperalgesic responses to IL-1β were inhibited in a dose-dependent manner by α-melanocyte stimulating hormone (α-MSH)-related peptides with the following order of potency: [N1[4],D-Phe[7]]α-MSH > α-MSH > Lys-D-Pro-Val > Lys-Pro-Val > Lys-D-Pro-Thr > D-Lys-Pro-Thr.

3 Hyperalgesic responses to PGE_2 were not inhibited by Lys-D-Pro-Thr and D-Lys-Pro-Thr but were inhibited in a dose-dependent manner by the other peptides with the same order of potency as against IL-1β.

4 The potencies of [N1[4], D-Phe[7]]α-MSH and α-MSH were greatly diminished by deletion of their C-terminal tripeptide, Lys[11]-Pro-Val[13].

5 Nor-binaltorphimine (Nor-BNI) largely reversed the analgesic effects of α-MSH, [N1[4], D-Phe[7]]α-MSH, Lys-Pro-Val and Lys-D-Pro-Val indicating that κ-opioid receptors mediated the analgesic activity of these peptides.

6 Nor-BNI did not antagonize the inhibition by Lys-D-Pro-Thr and D-Lys-Pro-Thr of IL-1β evoked hyperalgesia indicating that these peptides were not acting via κ-opioid receptors.

Keywords: Interleukin-1β; hyperalgesia; α-MSH; analgesic peptides

Introduction

The term interleukin-1 (IL-1) describes two pluripotent inflammatory proteins, IL-1α and IL-1β (March *et al.*, 1985), produced by activated macrophages and other cell types (Oppenheim *et al.*, 1986). In rats, recombinant human (rh) IL-1β is a potent hyperalgesic (nociceptive) agent that is inhibited by the tripeptide analogues of IL-1β, Lys[193]-Pro-Thr[195] and Lys-D-Pro-Thr (Ferreira *et al.*, 1988) and by α-melanocyte stimulating hormone (α-MSH) and its stable analogue [N1[4], D-Phe[7]]α-MSH (Follenfant *et al.*, 1989). α-MSH and [N1[4], D-Phe[7]]α-MSH inhibit other responses to IL-1 including fever and the production of acute-phase proteins (Daynes *et al.*, 1987; Robertson *et al.*, 1988). Also, the C-terminal tripeptide of α-MSH, Lys[11]-Pro-Val[13], is antipyretic against leucocyte pyrogen (Richards & Lipton, 1985), of which IL-1 is a major component (Dinarello, 1984), and is reported to have anti-inflammatory activity (Hiltz & Lipton, 1989; 1990).

To obtain information about the structure-activity relations of analgesic peptides we have tested the analgesic activities of a series of peptides related to α-MSH and Lys-D-Pro-Thr against the hyperalgesic agents IL-1β and prostaglandin E_2 (PGE_2, Ferreria, 1972). To investigate the possible involvement of κ-opioid receptors in the analgesic responses, the effects on the responses of the selective κ-opioid receptor antagonist, nor-binaltorphimine (nor-BNI, Portoghese *et al.*, 1987) were measured.

Methods

Hyperalgesia (nociception) in rats (Wistar, male, 135–170 g) was induced by IL-1β (0.1 international units, i.u.) or PGE_2 (100 ng) injected into the paw (intraplantar, i.pl.). Putative analgesic peptides were given intraperitoneally (i.p.) 1 h before injection of IL-1β and 2 h after injection of PGE_2. Nor-BNI was injected i.pl. at 5 μg/paw, 90 min before injection of IL-1β and 90 min after injection of PGE_2. There were five rats per treatment group and injection volumes were 0.1 ml and 0.3 ml for i.pl. and i.p. injections, respectively. Hyperalgesia was measured by a modification of the Randall-Selitto rat paw pressure test (Ferreria *et al.*, 1978; Nakamura & Ferreria, 1987). A constant pressure of 20 mmHg was applied to the hind paws of rats and discontinued when animals presented a characteristic freezing reaction (reaction time). The intensity of hyperalgesia was quantified as the variation of the reaction time (Δ reaction time) obtained by subtracting the value measured 3 h after administration of the hyperalgesic agent (IL-1β or PGE_2) from the pre-injection control reaction time (zero time). At this time interval after injection of the above doses of IL-1β and PGE_2, Δ reaction times were maximal (Ferreira *et al.*, 1988). The experimenter was unaware of the group treatments. Results are presented as means with s.e.mean ($n = 5$). ED_{50} values were calculated from $\log_e$ dose v. probit plots.

Materials

IL-1β produced in *E. coli* with a specific activity of 10^8 i.u. mg^{-1} and an endotoxin content of 0.25 endotoxin units/100,000 i.u. was the NIBSC preparation coded 86/680. PGE_2 was a gift from the Upjohn Co (U.S.A.) and Nor-BNI was a gift from Dr K. Rice, N.I.H. (U.S.A.). α-MSH and [N1[4], D-Phe[7]]α-MSH were purchased from Sigma (Poole, Dorset). α-MSH[1–10] and [N1[4], D-Phe[7]] α-MSH[1–10] were custom synthesized by Multiple Peptide Systems (San Diego, U.S.A). Lys-D-Pro-Thr, D-Lys-Pro-Thr, Lys-Pro-Val and Lys-D-Pro-Val were custom synthesized by Cambridge Research Biochemicals (Cambridge, England). The peptides were characterized by fast atom bombardment mass spectrometry, amino acid analysis and analytical reverse-phase high performance liquid chromatography (h.p.l.c.) by the manufacturers and purified to ⩾95% by preparative h.p.l.c. at NIBSC.

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Results

Interleukin-1β

IL-1β (0.1 i.u.) evoked hyperalgesia in rats: mean Δ reaction times were 21.2 ± 0.4 to 23.9 ± 0.5 s (Figures 1, 3 and 5). The inhibition of IL-1β-evoked hyperalgesia by eight peptides is shown in Figure 1 and ED_{50} values, with 95% confidence intervals, are given in Table 1. [Nl^4, D-Phe^7]α-MSH and α-MSH were the most potent inhibitors of the hyperalgesic effect of IL-1β and their potencies were greatly diminished by deletion of their C-terminal tripeptide, Lys^{11}-Pro-Val^{13} (KPV). Dose-response curves for the tripeptides Lys-D-Pro-Val (K(D)PV), Lys-Pro-Val (KPV), Lys-D-Pro-Thr (K(D)PT) and D-Lys-Pro-Thr ((D)KPT) lay to the right of curves for the larger peptides. Of the tripeptides, those with valine (V) at the C-terminus were 2–3 times more potent than those with threonine (T) in this position.

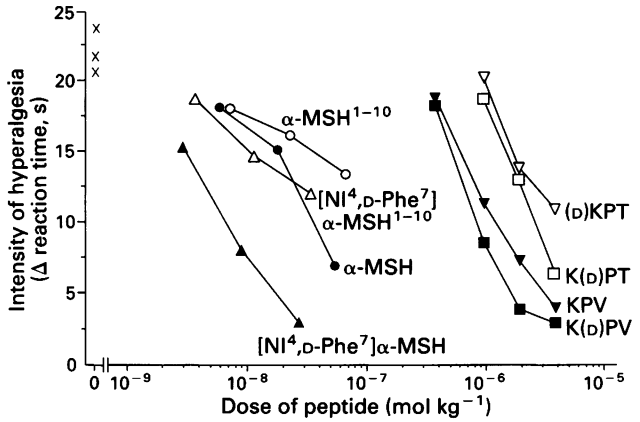

Figure 1 Hyperalgesic responses to interleukin-1β (IL-1β, 0.1 i.u., i.pl.) in rats treated with saline (X) and the inhibitory effects of eight peptides on the responses. The peptides (or saline) were injected (i.p.) 1 h before IL-1β. Symbols represent mean values in 5 rats; s.e.means were within the dimensions of the symbols. For abbreviations, see text.

Prostaglandin E_2

PGE_2 (100 ng) evoked hyperalgesia in rats: mean Δ reaction times were 16.8 ± 0.3 to 19.3 ± 0.5 s after PGE_2 (Figures 2, 4 and 5). The effects of eight peptides on PGE_2-evoked hyperplasia are shown in Figure 2 and Table 1, which gives ED_{50} values with 95% confidence intervals. Except for K(D)PT and (D)KPT the peptides inhibited PGE_2-evoked hyperalgesia. [Nl^4, D-Phe^7]α-MSH and α-MSH were the most potent peptides and their potencies were greatly diminished by deletion of their C-terminal tripeptide, Lys^{11}-Pro-Val^{13}. Dose-response curves for K(D)PV and KPV lay to the right of curves for the larger peptides. Approx 3 times higher doses of K(D)PV and KPV were required to inhibit PGE_2-evoked hyperalgesia than to inhibit IL-1β-evoked hyperalgesia.

Effect of nor-binaltorphimine on the analgesic activity of peptides against interleukin-1β

Inhibition by α-MSH and [Nl^4, D-Phe^7]α-MSH of IL-1β-evoked hyperalgesia was abolished by treatment with the

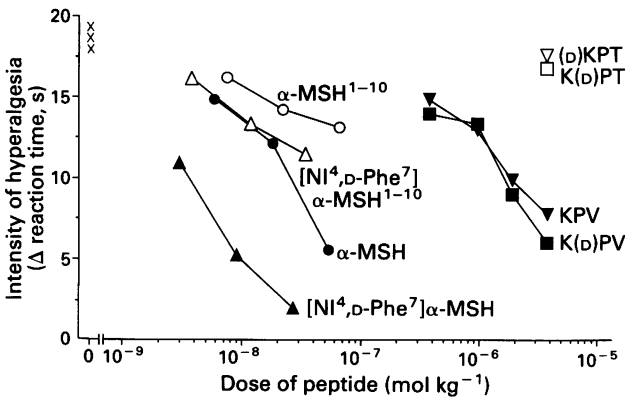

Figure 2 Hyperalgesic responses to prostaglandin E_2 (PGE_2, 100 ng, i.pl.) in rats treated with saline (X) and the effects of eight peptides on the responses. The peptides (or saline) were injected (i.p.) 2 h after PGE_2. Symbols represent mean values in 5 rats; s.e.means were within the dimensions of the symbols. For abbreviations, see text.

Table 1 Comparison of the inhibitory effects of eight peptides on the hyperalgesic effects of interleukin-1β (IL-1β, 0.1 i.u., i.pl.) and prostaglandin E_2 (PGE_2, 100 ng i.pl.)

Agonist	*Peptide inhibitor*	*ED_{50}* (mol kg^{-1})	*95% confidence interval* (mol kg^{-1})
IL-1β	[Nl^4,D-Phe^7]α-MSH	6.3×10^{-9}	3.0×10^{-9}–1.0×10^{-8}
	[Nl^4,D-Phe^7]α-MSH$^{1-10}$	4.5×10^{-8} [1]	—[2]
	α-MSH	3.2×10^{-8}	1.9×10^{-8}–8.0×10^{-8}
	α-MSH$^{1-10}$	2.0×10^{-8} [1]	—[2]
	K(D)PV	7.6×10^{-7}	4.4×10^{-7}–1.1×10^{-6}
	KPV	9.9×10^{-7}	5.9×10^{-7}–1.5×10^{-6}
	K(D)PT	2.1×10^{-6}	1.5×10^{-6}–3.3×10^{-6}
	(D)KPT	3.4×10^{-6}	2.4×10^{-6}–1.1×10^{-5}
PGE_2	[Nl^4,D-Phe^7]α-MSH	4.1×10^{-9}	1.2×10^{-9}–7.2×10^{-9}
	[Nl^4,D-Phe^7]α-MSH$^{1-10}$	7.0×10^{-8} [1]	—[2]
	α-MSH	2.5×10^{-8}	1.6×10^{-8}–7.0×10^{-8}
	α-MSH$^{1-10}$	4.6×10^{-7} [1]	—[2]
	K(D)PV	2.4×10^{-6}	1.5×10^{-6}–6.2×10^{-6}
	KPV	3.2×10^{-6}	1.8×10^{-6}–1.5×10^{-5}
	K(D)PT	—[3]	—
	(D)KPT	—[3]	—

The peptides (or saline) were injected (i.p.), 1 h before IL-1β and 2 h after PGE_2., ED_{50} values and 95% confidence intervals were calculated from $\log_e$ dose v. probit plots with the assumption that the peptides had the capacity to reverse fully the hyperalgesic responses.

[1]Crude estimates based upon relatively flat slopes for dose-response curves suggesting an inability of these compounds to reverse fully hyperalgesic responses.

[2]Flat slopes for dose-response curves precluded calculation of meaningful confidence intervals.

[3]At 1.3 mg kg^{-1} (= 3.8 μmol kg^{-1}) the peptides did not inhibit PGE_2-evoked hyperalgesia.

For abbreviations, see text.

κ-opioid antagonist, Nor-BNI (5 μg, i.pl., Figure 3). In contrast inhibition by α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ of IL-1β-evoked hyperalgesia was only partially attenuated by Nor-BNI (Figure 4) and there remained a marginal regression with dose of α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ ($0.01 < P < 0.2$) in the presence of Nor-BNI.

Nor-BNI effectively abolished inhibition of IL-1β-evoked hyperalgesia by K(D)PV and KPV but had no effect on inhibition by K(D)PT and (D)KPT (Figure 5).

Effect of nor-binaltorphimine on the analgesic activity of peptides against prostaglandin E_2

Inhibition by α-MSH and [Nl^4, D-Phe^7]α-MSH of PGE_2-evoked hyperalgesia was abolished by treatment with Nor-BNI whereas inhibition by α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ was only partially attenuated by Nor-BNI (Figure 4) and there remained marginal regression with dose of α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ ($0.01 < P < 0.2$) in the presence of Nor-BNI.

Nor-BNI effectively abolished inhibition of PGE_2-evoked hyperalgesia by K(D)PV and KPV but did not alter the lack of effect of K(D)PT and (D)KPT on responses to PGE_2.

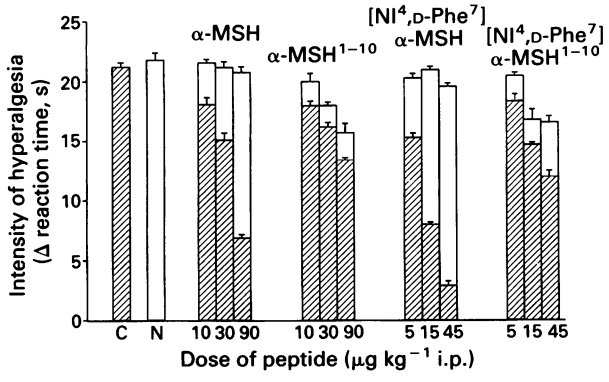

Figure 3 Hyperalgesic responses to interleukin-1β (IL-1β, 0.1 i.u., i.pl.) in rats treated with saline (hatched columns) or nor-binaltorphimine (Nor-BNI, 5 μg, i.pl., open columns) and the inhibitory effects of α-MSH, [Nl^4, D-Phe^7]α-MSH, α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ on the responses. Nor-BNI was injected 90 min before IL-1β; peptides were injected (i.p.) 1 h before IL-1β. C = rats injected with saline/IL-1β; N = rats injected with Nor-BNI/IL-1β. The vertical bars are s.e.means of values obtained in groups of 5 rats. For abbreviations, see text.

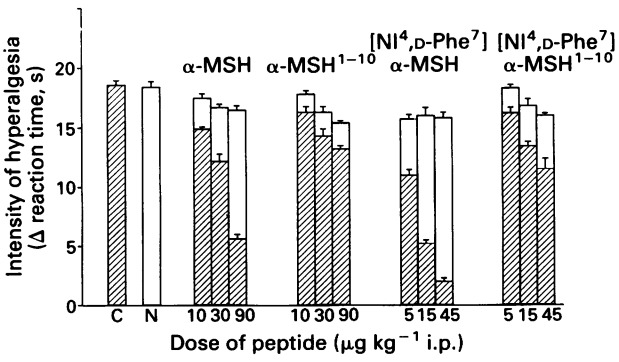

Figure 4 Hyperalgesic responses to prostaglandin E_2 (PGE_2, 100 ng, i.pl.) in rats treated with saline (hatched columns) or nor-binaltorphimine (Nor-BNI, 5 μg, i.pl., open columns) and the inhibitory effects of α-MSH, [Nl^4,D-Phe^7]α-MSH, α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ on the responses. Nor-BNI was injected 90 min after PGE_2; peptides were injected (i.p.) 2 h after PGE_2. C = rats injected with saline/IL-1β; N = rats injected with Nor-BNI/IL-1β. The vertical bars are s.e.means of values obtained in groups of 5 rats. For abbreviations, see text.

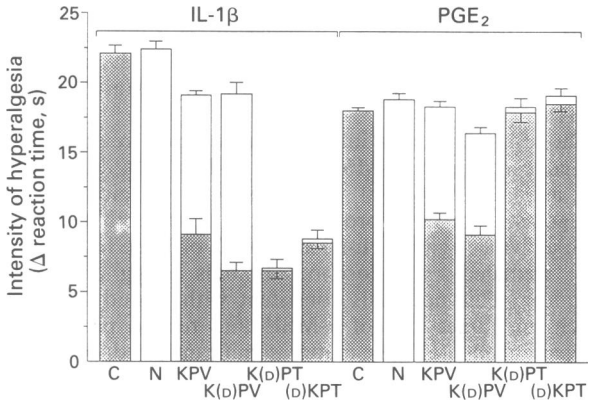

Figure 5 Hyperalgesic response to interleukin-1β (IL-1β, 0.1 i.u., i.pl.) or prostaglandin E_2 (PGE_2 100 ng, i.pl.) in rats treated with saline (hatched columns) or nor-binaltorphimine (Nor-BNI, 5 μg, i.pl., open columns) and the effects of 4 tripeptides (3.8 μmol kg^{-1}, i.p.) on the responses. Nor-BNI was injected 90 min before IL-1β and 90 min after PGE_2; peptides were injected 1 h before IL-1β and 2 h after PGE_2. C = rats injected with saline/IL-1β; N = rats injected with Nor-BNI/IL-1β. The vertical bars are s.e.means of values obtained in groups of 5 rats. For abbreviations, see text.

Discussion

α-MSH and its stable analogue [Nl^4, D-Phe^7]α-MSH (Sawyer *et al.*, 1980) inhibit a number of biological responses to IL-1 including fever, increased plasma concentrations of acute phase proteins and corticosterone, numbers of circulating neutrophils (Daynes *et al.*, 1987) and hyperalgesia (Follenfant *et al.*, 1989). Except for IL-1-evoked proliferation of thymocytes (Cannon *et al.*, 1986), [Nl^4, D-Phe^7]α-MSH has been found to be a more potent inhibitor than the natural hormone. In the present study [Nl^4, D-Phe^7]α-MSH was the most potent of the peptides tested, with an ED_{50} value for inhibition of IL-1β-evoked hyperalgesia of 6.3 nmol kg^{-1}, similar to that, 9.0 nmol kg^{-1}, reported by Follenfant *et al.* (1989). The ED_{50} values of α-MSH (3.2×10^{-8} mol kg^{-1}) and Lys-D-Pro-Thr (2.1×10^{-6} mol kg^{-1}) also were very similar to the values obtained in the earlier study.

The marked loss in analgesic activity that resulted from deletion of the C-terminal peptide, Lys^{11}-Pro-Val^{13}, from α-MSH and [Nl^4, D-Phe^7]α-MSH together with the analgesic activity of the tripeptide show its importance to the analgesic activities of α-MSH and its stable analogue. This finding is consistent with the work of Deeter *et al.* (1989), who tested a series of α-MSH analogues with truncated N-termini for antipyretic activity against IL-1 and found the order of potency to be α-MSH > α-MSH$^{8-13}$ > α-MSH$^{11-13}$ > α-MSH$^{10-13}$. The residual activity in α-MSH$^{1-10}$ and [Nl^4, D-Phe^7]α-MSH$^{1-10}$ suggests an additional site with analgesic activity within these decapeptides. The increases in analgesic activity resulting from the substitution of D-Pro for L-Pro in Lys-Pro-Thr (Ferreira *et al.*, 1988) and Lys-Pro-Val suggest that a similar substitution made in the stable analogue of α-MSH, giving [Nl^4, D-Phe^7,D-Pro^{12}]α-MSH, would yield a potent and long-lasting analgesic agent.

Of the peptides studied only one, Lys-D-Pro-Thr, had been tested previously for inhibitory activity against hyperalgesia evoked by PGE_2, with a negative result (Ferreira *et al.*, 1988; Follenfant *et al.*, 1989). This finding was confirmed and D-Lys-Pro-Thr also was found not to have analgesic activity against PGE_2. In contrast, the six other peptides tested had analgesic activity against PGE_2 with the same order of potency as against IL-1β. The capacity of Lys-Pro-Val and Lys-D-Pro-Val but not Lys-D-Pro-Thr and D-Lys-Pro-Thr to inhibit PGE_2-evoked hyperalgesia suggests that the analgesic effect of the threonine compounds might be specific to IL-1β although there is no evidence that these tripeptides compete

for IL-1 receptors since Lys-D-Pro-Thr was not an antagonist of IL-1β-evoked fever or stimulation of EL-4 mouse thymoma cells (Ferreira *et al.*, 1988) or of IL-1-evoked relaxation of rabbit isolated mesenteric artery (Marceau *et al.*, 1991).

The marked attenuation or near complete reversal of the analgesic effects of α-MSH, [N1[4], D-Phe[7]]α-MSH, Lys-Pro-Val and Lys-D-Pro-Val by the specific κ-opioid receptor antagonist, Nor-BNI, provides good evidence that the analgesic activities against IL-1β and PGE_2 of these four peptides were mediated by endogenous opioids acting on κ-opioid receptors. This notion is consistent with a study in rats in which microinjections of α-MSH and related peptides into the periaqueductal grey matter significantly reduced responsiveness to pain, with α-MSH eliciting a behavioural profile similar to that produced by β-endorphin (Walker *et al.*, 1980). Similarly, in mice, the opioid receptor antagonist, naloxone, (given subcutaneously) prevented the analgesic action of α-MSH (given intracerebroventricularly, Ohkubo *et al.*, 1984). The partial reduction in the analgesic activity of $\alpha\text{-MSH}^{1-10}$ and [N1[4], D-Phe[7]]$\alpha\text{-MSH}^{1-10}$ in the presence of Nor-BNI suggest that the residual analgesic activities of these peptides (present after deletion of Lys^{11}-$Pro\text{-}Val^{13}$) was only in part mediated by κ-opioid receptors. The failure of Nor-BNI to diminish the analgesic effects of Lys-D-Pro-Thr and D-Lys-Pro-Thr indicates that these peptides were not working via κ-opioid receptors. This finding is consistent with the lack of effect of the opioid receptor antagonist, naloxone, on inhibition by Lys-D-Pro-Thr of IL-1β-evoked hyperalgesia (Follenfant *et al.*, 1989). Also consistent with the non-opioid mechanism of action of Lys-D-Pro-Thr is its failure to antagonize PGE_2-evoked hyperalgesia (Ferreira *et al.*, 1988), in contrast to morphine (Lorenzetti & Ferreira, 1985).

Although α-MSH has been shown to have analgesic activity when given centrally (Walker *et al.*, 1980; Ohkubo *et al.*, 1984) it lacked effect on PGE_2-evoked hyperalgesia when given i.v. in a hot-plate test, in contrast to morphine (Follenfant *et al.*, 1989). Similarly Lys-D-Pro-Thr, Lys-Pro-Val and Lys-D-Pro-Val were not effective in a hot-plate test, in contrast to morphine (Ferreira *et al.*, 1988; Ferreira: unpublished data). These data suggest that the peptides tested in the present study had a peripheral rather than a central site of action.

The authors thank Ms I.R. Santos and Mrs. S. Selkirk for technical assistance. This work was supported by British Technology Group (UK) and FAPESP, FINER, CNPq (Brazil).

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(Received November 19, 1991
Revised February 21, 1992
Accepted February 27, 1992)