# C-terminal fragment (residues 61–91) of β-lipotropin: Is it the natural opiate-like neurohormon of the brain?

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*Summary.* The C-terminal fragment of porcine β-lipotropin (residues 61–91) has a very strong analgesic activity in vivo in rats. It is 20 times more potent than morphine if administered intracerebroventricularly (ICV) to rats. Its effect could be antagonized by naloxone. Presumably it is a natural opiate-like neurohormon of the brain.

An endogenous peptide with opioid agonist activity termed enkephalin was isolated by HUGHES et al.[1] and more recently the primary structure of this pentapeptide has been elucidated: H–Tyr–Gly–Phe–$\overset{\text{Met}}{\underset{\text{Leu}}{}}$–OH[2]. This pentapeptide mimicks the in vitro activity of morphine to block the electrically evoked contractions of mouse vas deferens and guinea-pig ileum, furthermore it inhibits the stereospecific binding of opiate antagonist $^3$H-naloxone in brain homogenates[2]. It has been proposed that the opioid agonists exert their analgesic action at the enkephalin receptors and the enkephalin itself may be the modulator or transmitter for pain suppression[3,4]. However in previous experiments we could not detect significant and sustained analgesia following Met-enkephalin administration, i.e. its in vivo efficacy was not comparable with that of the morphine[5,6], while BELUZZI et al.[7] reported on a short-lasting analgesic effect, which could be antagonized by naloxone. The amino acid sequence of enkephalin is contained in the primary structure of β-lipotropin between amino acid residues 61–65[8,9]. According to our previous experiments, β-lipotropin preparation showed a weak morphine-like in vivo activity[5,6] suggesting that another part of lipotropin molecule may be responsible for the in vivo analgesic effect. Therefore we analyzed some C-terminal fragments of β-lipotropin for analgesia. The experiments were focussed primarily on a fragment containing residues 61–91 (LPH-[61–91]), because this terminal fragment of β-lipotropin was found to be more active than enkephalin by LI and CHUNG[10] and BRADBURY et al.[11] as tested in vitro in receptor binding assay. (This naturally occurring fragment was designated as β-endorphin by LI and CHUNG[10].)

*Materials and methods.* LPH-(61–91) was isolated from a crude porcine adrenocortocotropic hormone preparation[12] by high voltage paper electrophoresis at pH 6,5. The peptide was characterized by *N*-terminal residue[13] and amino acid analysis and also by mapping of its tryptic digest. The analgesic effect of substances was tested by the tail-flick method in rats[14], a procedure with good selectivity for opiate drugs. Male rats weighing 120–130 g were used. The intensity of heat irradiation was regulated such that the control reaction time of tail-withdrawal – measured automatically – varied between 3 and 6 sec. A cutoff time of 15 sec was applied. In the quantal statistical assay, the animals were considered positive for analgesia if the reaction time was prolonged

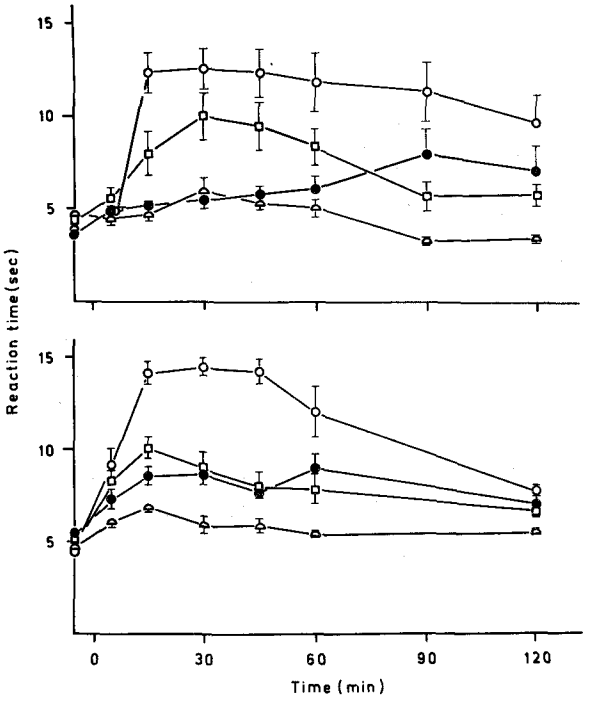


Fig. 1. Time-response curves of the analgesic effects of morphine (upper panel) and LPH-(61–91) (lower panel), given intracerebroventricularly to rats. On the abscissa is plotted the time elapsed after ICV administration of drugs expressed in minutes. The ordinate shows the reaction time in seconds. The symbols are as follows:

Upper panel: ○ morphine $1.68\times10^{-8}$ mole/animal ($n$ = 10);
□ morphine 8.4 $\times10^{-9}$ mole/animal ($n$ = 15);
⌂ morphine 4.2 $\times10^{-9}$ mole/animal ($n$ = 10);
● morphine 6.8 $\times10^{-8}$ mole/animal + naloxone. 2 mg/kg s.c. 30 min prior to morphine ($n$ = 10).

Lower panel: ○ LPH-(61–91) $1.6\times10^{-9}$ mole/animal ($n$ = 12);
□ LPH-(61–91) $4.0\times10^{-10}$ mole/animal ($n$ = 12);
⌂ LPH-(61–91) $2.0\times10^{-10}$ mole/animal ($n$ = 12);
● LPH-(61–91) $1.6\times10^{-9}$ mole/animal + naloxone. 2 mg/kg s.c. 30 min prior to LPH-(61–91) ($n$ = 12).

Saline (20 µl ICV) or saline + naloxone (2 mg/kg nalosone s. c. 30 min before saline administration) failed to alter significantly the reaction time.

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for at least twice the control value. The substances were dissolved in 20 µl saline and injected directly into the right lateral horn of the third ventricle according to CHERMAT and SIMON[15]. The doses of the compounds administered ICV were expressed in mole/animal.

*Results.* The time-response curves showing the analgesic effect of morphine and LPH-(61–91) are of similar character (Figure 1). The analgesic effect developed gradually, reached the maximum between 30 and 60 min and was sustained for hours. The slow onset of morphine's action when administered intracerebrally was also shown by others[16,17]. The effect of both morphine and LPH-(61–91) could be antagonized by naloxone, given subcutaneously 30 min prior to ICV administration of the compounds (Figure 1). The reaction times in the morphine or LPH-(61–91) + naloxone treated group were significantly lower ($p < 0.05$) than in the animals given morphine or LPH-(61–91) alone, at 15, 30, 45 and 60 min and 5, 15, 30 and 45 min, respectively. As the Figure 2 shows, the dose-response curves plotted from the data transformed for probit analysis[18] are parallel. The $ED_{50}$ values were $7.7 \times 10^{-9}$ (5.4–11.0) mole/animal for morphine and $4 \times 10^{-10}$ (2.7–5.9) mole/animal for LPH-(61–91), i.e. the potency ratio was as high as 19.3 (11.3–32.7) calculated on molar basis. The Met-enkephalin and LPH-(61–79) also showed weak analgesic action (the latter being the more efficient) but their activity was too low for calculating the $ED_{50}$ values tested at doses as high as $6.7 \times 10^{-7}$ and $8 \times 10^{-8}$ mole/animal, respectively.

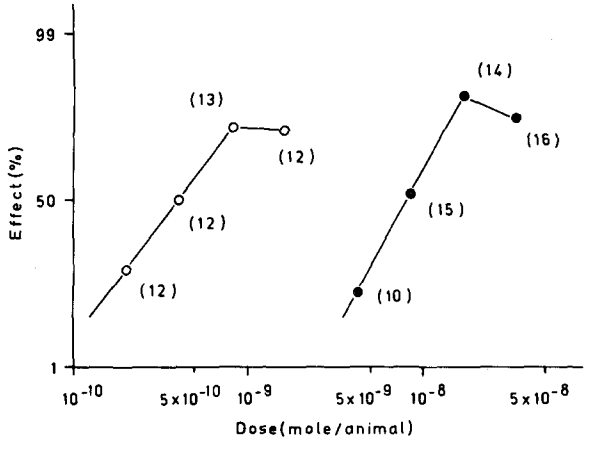


Fig. 2. Dose-response curves of the analgesic effect of morphine (●) and LPH-(61–91) (○), plotted according to LITCHFIELD and WILCOXON[18].

*Discussion.* According to the data in the literature, a β-LPH fragment, to exhibit opiate agonist properties in vitro, must contain residues 61–65 at the *N*-terminus. However, this segment is not sufficient to produce remarkable in vivo efficacy. For the latter, the whole C-terminal fragment of β-LPH is required. It may be speculated that LPH-(61–91) contains further binding site(s) within residues 66–91 and/or this part of the molecule has a protective role against the inactivating enzyme(s) of the brain. In any case, it seems probable that the LPH-(61–91) is a (the ?) physiological modulator in mammalian brain in supression of the pain reaction.

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## 7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide, a new antihypertensive agent

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*Summary.* 7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide (DU-717) is a new compound having sustained antihypertensive activity in a similar manner to that of hydrochlorothiazide. However, this compound shows neither diuretic nor hyperglycemic effect, being different from those of hydrochlorothiazide or diazoxide.

During the process of pharmacological studies on a series of 1,2,4-benzothiadiazine-1,1-dioxide derivatives, we have found that some compounds show an antihypertensive activity without diuretic and hyperglycemic effect in experimental animals. 7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide (DU-717) is one of such compounds with the chemical structure shown in figure 1.

Hypotensive activity was investigated in male spontaneously hypertensive rats (SHR)[1], 12–16 weeks of age and 270–320 g of body weight, with systolic blood pressure levels above 170 mm Hg, or in normotensive Wistar rats (NWR), 300–350 g of body weight, with systolic blood pressure levels 120–140 mm Hg. DU-717, diazoxide and hydrochlorothiazide (HCT) were suspended in 0.5% tragacanth solution and administered orally by a stomach tube. Blood pressure was measured with the tail-plethysmographic method after warming the animal in a heated box maintained at 38 °C for 10 min without anesthesia. Hypotensive activity of a single administration was investigated in SHR and blood pressure was measured prior to dosing and 1, 3, 5, 7 and 24 h after an oral administration. DU-717 and HCT at a dose of 1000 mg/kg showed no effect on blood pressure. However, diazoxide at a dose of 100 mg/kg significantly lowered blood pressure and the maximum effect was observed 5 h after an oral administration. Moreover, hypotensive activity of a repeated administration was investigated in SHR and NWR. DU-717, diazoxide and HCT were administered orally to SHR and NWR once a day for 10 successive days. Blood pressure was measured prior to dosing and 5 h after an oral administration on alternate days. At a dose of 1 mg/kg/day, DU-717, diazoxide and HCT showed

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