β-Endorphin Fragments DTγE and DEγE Reduced Morphine Inhibition of Electrically-Induced Contractions and Opiate Withdrawal

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Abstract: The effect exerted by two γ-endorphin derivatives (DTγE and DEγE) was investigated on morphine-induced inhibition on the electrically induced contractions of guinea pig ileum *in vitro*. Morphine ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$ M) dose dependently and significantly reduced the E.C. of guinea pig ileum, IC50=$6.5x10^{-8}$ M (Confidence limits: $3.7x10^{-8}$-$9.1x10^{-8}$). DTγE and DEγE *per se* ($1x10^{-6}$-$5x10^{-6}$-$1x10^{-5}$ M) did not modify significantly the E.C. of guinea pig ileum. Furthermore, DTγE or DEγE injection 10-30-60 min before morphine, did not affect the inhibitory effect of morphine on the E.C. of guinea pig ileum. By contrast, ilea from guinea-pigs treated for 4 days with DTγE or DEγE (1 mg/Kg/i.p.) were less sensitive to the inhibitory effect of morphine, IC50=$8.3x10^{-7}$ M (Confidence limits: $1.4x10^{-6}$-$3.5x10^{-7}$) for DTγE and IC50=$7.7x10^{-7}$ M (Confidence limits: $2.7x10^{-6}$-$8.7x10^{-7}$) for DEγE .

The effect exerted by two β-endorphin fragments (DTγE and DEγE) was investigated on the acute opiate withdrawal induced by μ, k and δ receptor agonists *in vitro*.

After a exposure *in vitro* for 4 min to morphine (less selective μ agonist), DAGO (highly selective μ agonist), U50-488H (highly selective k agonist) and β-endorphin (selective μ-δ agonist), a strong contracture of isolated guinea pig ileum was observed after the addition of naloxone. This effect was also observed when isolated rabbit jejunum was pretreated with deltorphin (highly selective δ agonist).

DTγE or DEγE injection before or after treatment with morphine, DAGO, U50-488H, β-endorphin or deltorphin was able of both preventing and reversing the naloxone-induced contracture after exposure to the opioid agonists in a concentration-dependent fashion.

Our results indicate that both DTγE or DEγE are able to reduce significantly opiate withdrawal *in vitro,* suggesting an important functional interaction beween β-endorphin fragments and opioid withdrawal at both μ, k and δ receptor level.

Our results indicate that chronic treatment of guinea pigs with DTγE or DEγE induces a significant reduction of the inhibitory effect of morphine on the E.C. of guinea-pig ileum thus confirming an important functional interaction between γ-endorphin derivatives and opioid system.

Key Words: β-endorphin fragments, electrical stimulation, guinea pig ileum, opioid withdrawal.

INTRODUCTION

It has been suggested that the β-lipotropin fragment DTγE (β-LPH_{62-77}) may have neuroleptic-like effecs in laboratory animals [1]. Even though it showed antipsychotic activity in a number of schizophrenic patients [2, 3] it could be regarded as a classical neuroleptic compound [4]. In view of these findings, many studies have focused on the possible interference exerted by DTγE and DEγE on the brain dopaminergic system [5-7]. On the contrary, few data are available on the effects exerted by DTγE and DEγE on the opioid system. Previous data show that removal of the N-terminal amino acid residue tyrosine from γ-endorphin resulting in DTγE leads to a loss of opioid activity, with a loss of capacity to interfere with opioid receptors [1, 8, 9]. On the other hand, Neil *et al*. [10] reported that chronic systemic DTγE treatment in rats increased morphine analgesia, resembling the effect observed after chronic naloxone exposure [11, 12].

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Our laboratory recently started an investigation on the possible interference exerted by the β-endorphin fragments on some effects exerted by centrally-acting drugs or spontaneous brain excitability [13-15]. Recently, it was demonstrated that DTγE was able to reduce dose-dependently the analgesic effect induced by morphine in mice probably through an indirect mechanism [8].

The aim of this work was to further study the influence of the β-endorphin fragments, DTγE and DEγE, on the opioid system by using electrically stimulated-guinea pig ileum which is an established model for opiate receptor studies [16] and their effects on opioid withdrawal *in vitro* [17-21]. Therefore, the author considered

1. the effects of acute and chronic DTγE and DEγE pretreatment on the inhibitory effect induced by morphine on the E.C. of guinea pig ileum in order to assess whether these β-endorphin fragments modify morphine effects through a direct or indirect interaction with opioid receptors.

2. the effects of DTγE and DEγE on the acute dependence induced by morphine, DAMGO and U50-488H by using the

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isolated guinea pig ileum in which μ and k opioid receptors are widely distributed. The second set of experiments was performed on the isolated rabbit jejunum which contains δ opiate receptors.

MATERIALS AND METHODS

Animals

Male Charles River guinea-pigs (180-200 g) were used for all the experiments. The animals were housed in colony cages (4 guinea-pig each) under conditions of standard light (lights on from 7.00 a.m. to 7.00 p.m.), temperature (22±1°C) and room humidity (60%±10%) conditions for at least 1 week before the experimental sessions. Food and water were available *ad libitum*.

Transmurally Stimulated Guinea-Pig Ileum Test

Guinea-pig ileum was prepared as described previously [22]. The animals were sacrificed by CO_2 and bled. Pieces of ileum, 2-3 cm long were set up in a 10 ml organ bath containing Tyrode solution with 5% CO_2 in 95% oxygen: the solution was maintained at 37°C. Whole ileum preparation was placed between platinum electrodes and connected to a 85/2/50 M.A.R.B. Stimulator (DITTA M.A.R.B. Chiesina Uzzanese, Pistoia, Italy). An isotonic transducer and unirecord model poligraph was used for measurements of isotonic contractions (Ugo Basile, Italy). A resting tension of 0.5 g was applied. After an equilibration period of 30 min, the preparation was stimulated for 0.5 msec pulse delivered transmurally at a frequency of 10 sec at sopramaximal voltage (25V). In these conditions, the preparation showed a contraction mean of 60 mm±57. The inhibition of ileal contractions by drugs was expressed as percentage of basal value (mean±S.E.M.). Some animals (n=9) were treated with DEγE or DTγE (1 mg/Kg/i.p.) or with distilled water (10 ml/kg/i.p.) for 4 days.

Experimental Procedure

In order to assess a possible influence of peptidase enzymes on DTγE and DEγE activity, in some preliminary experiments, we added to Tyrode solution, an inhibitor of peptidase, bestatin, at a concentration of 10^{-4} M. No difference was observed in DTγE and DEγE activity with or without bestatin. Therefore, all experiments were performed without bestatin. DTγE and DEγE were dissolved in distilled water and their effects on morphine-induced inhibition on E.C. of guinea pig ileum were investigated according to the following experimental schedule:

a) DTγE or DEγE $1x10^{-6}$-$5x10^{-6}$-$1x10^{-5}$ M : 10-30-60 min before morphine injection ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$ M).

b) Morphine ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$ M) on ilea treated with DTγE or DEγE (1 mg/Kg/i.p. for 4 days).

Acute Opiate Dependence on Isolated Guinea-Pig Ileum

The animals were killed by $C0_2$ and bled. The terminal portion of the ileum, after discarding the 10 cm segment nearest the caecum, was kept in a Petri dish with Tyrode solution (g/l: NaCl 8.00, KCl 0.20, $CaC1_2$ 0.20, $MgC1_2x$ $6H_20$ 0. 10, $NaH_2P0_4xH_2O$ 0.05, $NaHC0_3$ 1.00, Glucose 1.00) for 30 min and then washed of free faecal matter. Segments, 2-3 cm long, from the same animal were placed between platinum electrodes and connected to a 85/2/50 model M.A.R.B. Stimulator (Ditta M.A.R.B., Chiesina Uzzanese, Pistoia, Italy). A force-displacement transducer and unirecord model polygraph was used for measurement of isotonic contractions (Ugo Basile, Milano, Italy). A resting tension of 0.5 g was applied. The baths were maintained at 37°C and continuously bubbled with a mixture of 95% 0_2 and 5% $C0_2$.

The experimental procedure was the same as that described previously [22]. The ilea were allowed to equilibrate for 40-60 min without washing and the response to acetylcholine (10^{-6} M) was determined three times so that responses could be expressed as percentage of acetylcholine maximum response. A reproducible acute opiate dependence was obtained after performing the following experimental procedure. A typical tracing of contracture responses of the ileum to repeated challenges with opiate and naloxone is shown in (Fig. **1**). After three similar acetylcholine responses, the preparation was electrically stimulated for 10-20 min

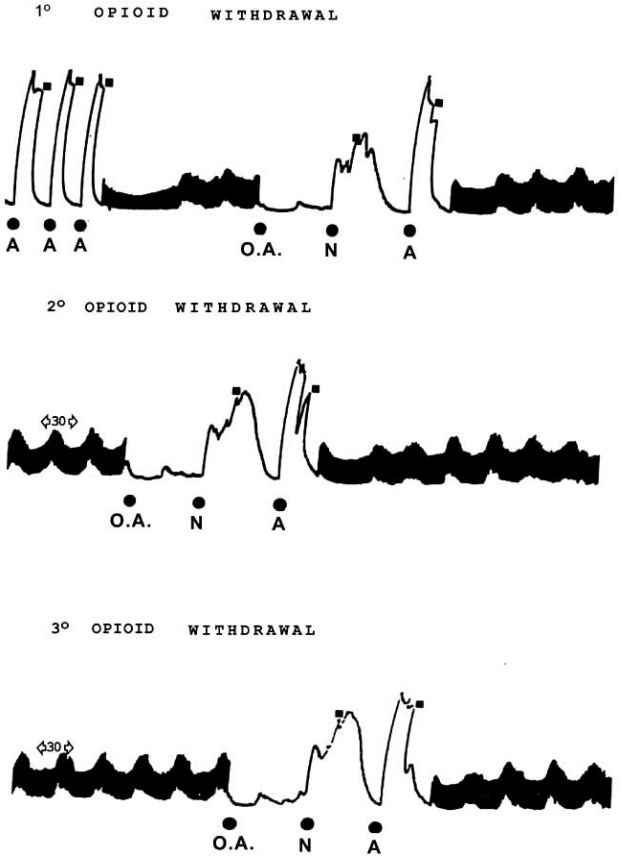

Fig. (1). Typical tracing of opioid withdrawal on guinea-pig ileum. A. 3 similar acetylcholine responses (A), electrical stimulation, injection of the opioid agonist (OA) followed, after 4 min of contact period, by naloxone (N) which induces contraction (1° opioid withdrawal). After washout (▮), it was followed by another A response.

B: After 30 min resting period under electrical stimulation, a further 4 min exposure of the ileum to the OA and N elicited reproducible response (2° opioid withdrawal).

C: After another 30 min resting period under electrical stimulation, the ileum responded again to the OA and N with the same intensity (3° opioid withdrawal).

(0.5 ms pulse delivered transmurally, at a frequency of 0.1 Hz at supramaximal voltage 25 V). Before addition of opioid agonist to the bath, the electrical stimulation was switched off. Under these conditions, the first contact with the opioid agonist followed after exposure to naloxone for 4 min induced a strong contracture (about 60% of the acetylcholine maximum) (Fig. **1A**). However, after washout, another acetylcholine response was performed (to verify whether the ileum responsiveness was modified after withdrawal contracture), and, after a resting period of 30 min under stimulation, a further 4-min exposure of the ileum (without electrical stimulation) to the opiate and naloxone elicited a reproducibile response (Fig. **1B**). Following washout, acetylcholine response (Fig. **1B**) and another 30 min resting period under stimulation, the ileum responded again to the opiate agonist and naloxone with the same intensity (Fig. **1C**). In our experiments, to avoid a possible tolerance due to repeated opiate injections, each preparation was submitted only to three challenges with the opioid agonist and naloxone.

Naloxone *per se* did not produce effects on "naive" preparations or those washed after opiate contact.

Acute Opiate Dependence on Rabbit Isolated Jejunum

The experimental procedure was descibed previously [22, 23]. Briefly, the animals were sacrificed by CO_2 and bled. The abdomen was opened by midline incision and segments of jejunum 3 cm long were removed from the same animal and placed in 10 ml tissue baths containing Tyrode solution. The tissues were connected to an isotonic transducer counter balanced by 1g loading and allowed to equilibrate for 45 min; during this period regular spontaneous activity was recorded. Deltorphin, a δ selective receptor agonist, was used to induce acute dependence. The opiate dependence procedure was the same as the one performed on the isolated guinea pig ileum. On the other hand, in this case, electrical stimulation was not used because the jejunum *per se* possesses a marked spontaneous activity very similar to that induced by electrical stimulation on other preparations, i.e., guinea pig ileum.

Experimental Procedure

The administration of DTγE and DEγE was performed according to the following schedule:

a) 3 acetylcholine responses

b) electrical stimulation (10-20 min)

c) administration of opiate agonists in absence of electrical stimulation (4 min) and addition of naloxone with subsequent contraction (1° opioid withdrawal)

d) washout and acetylcholine response

e) electrical stimulation (30 min)

f) DTγE or DEγE ($1x10^{-9}$-$5x10^{-8}$-$1x10^{-8}$ M) without electrical stimulation, added 10 min before or after the opioid agonist followed by naloxone (2° opioid withdrawal)

g) washout and acetylcholine response

h) electrical stimulation (30 min)

i) final control opiate withdrawal (3° opioid withdrawal).

In our experimental conditions to induce a strong contracture, each opioid agonist and naloxone were administered at the following concentrations: Morphine (10^{-5} M) + Naloxone (10^{-5} M); DAGO (10^{-6} M) + Naloxone (10^{-6} M); U50-488H (10^{-8} M) + Naloxone (10^{-5} M); β-endorphin (10^{-8} M) + Naloxone (10^{-5} M); Deltorphin (10^{-8} M) + Naloxone (10^{-5} M)

Each experiment was performed on at least 6 to 9 isolated preparations from different animals.

Drugs

All drugs used in the experimental sessions were purchased from Sigma Chemical Co. (St. Louis, MO, USA) with the exception of morphine HCl, which was purchased from Carlo Erba (Milan, Italy), DEγE was kindly donated by Organon International BV, Oss, The Netherlands.

Parameter Evaluation

Four parameters were evaluated:

1) Naloxone contracture: the size of the contracture produced by the naloxone challenge was expressed as a fraction of the maximum contraction obtained with the subsequent addition of acetylcholine in the same piece of tissue according to a modification of the method of Collier *et al*., [19]:

$$\frac{\text{Response to naloxone}}{\text{Maximum response to Ach}} \times 100 = \text{Tension ratio}$$

2) Acetylcholine responses before and after treatment: reduction or increase of the acetylcholine responses in the post-drug was expressed as a percentage of the acetylcholine response in the pre-drug period.

3) Electrically stimulated contraction before and after treatment: reduction or increase of the contraction following electrical stimulation contraction in the post-drug period was expressed as a percentage of the electrical stimulation contraction in the pre-drug period.

4) Naloxone contraction before and after treatment: reduction or increase of the naloxone contraction in the post-drug period was expressed as a percentage of the naloxone-induced contraction in the pre-drug.

Statistical Analysis

Regression methods were used for statistical analysis and critical significance set at $P<0.05$ for transmurally stimulated guinea-pig ileum test. Results of opiate withdrawal were expressed as mean±S.E.M. and tested for statistical significance using Student's t-test for paired data when results before and after treatments on the same preparation were compared.IC50 values and confidence limits (95%) were calculated with the method reported by Tallarida and Murray [24].

RESULTS

Transmurally Stimulated Guinea-Pig Ileum Test

The effect exerted by two γ-endorphin derivatives (DTγE and DEγE) was investigated on morphine-induced inhibition on the -induced contractions of guinea pig ileum *in vitro*.

Morphine ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$ M) dose dependently and significantly reduced the E.C. of guinea pig ileum, IC50= $6.5x10^{-8}$ M (Confidence limits: $3.7x10^{-8}$-$9.1x10^{-8}$) (Table **1**). DTγE and DEγE *per se* ($1x10^{-6}$-$5x10^{-6}$-$1x10^{-5}$ M) did not significantly modify the E.C. of guinea pig ileum (Table **1**). Furthermore, DTγE or DEγE injection 10-30-60 min before morphine did not affect the inhibitory effect of morphine on the E.C. of guinea pig ileum (Table **1**). By contrast, ilea from guinea-pigs treated for 4 days with DTγE or DEγE (1 mg/Kg/i.p.) were less sensitive to the inhibitory effect of morphine, IC50=$8.3x10^{-7}$ M (Confidence limits: $1.4x10^{-6}$-$3.5x10^{-7}$) for DTγE and IC50=$7.7x10^{-7}$ M (Confidence limits: $2.7x10^{-6}$-$8.7x10^{-7}$) for DEγE (Table **1**). The effect of morphine was not modified in the ilea from guinea-pigs treated for 4 days with distilled water (10 ml/kg/i.p.) (Table **1**).

Table 1. IC50 and 95% C.I. Values of of DTγE and DEγE on Morphine-Induced Inhibition on the Electrically-Induced Contractions of Guinea Pig Ileum *In Vitro*. DTγE and DEγE were Administered

Treatment	IC_{50} (95% C.I, Values)
Morphine	$6.5x10^{-8}$ M ($3.7x10^{-8}$-$9.1x10^{-8}$).
DTγE (acute) + Morphine	$6.7x10^{-8}$ M ($2.9x10^{-8}$-$8.7\ x10^{-8}$).
DEγE (acute) + Morphine	$6.3x10^{-8}$ M ($2.5x10^{-8}$-$7.9x10^{-8}$).
DTγE (4 days) + Morphine	$8.3x10^{-7}$ M ($1.4x10^{-6}$-$3.5x10^{-7}$)
DEγE (4 days) + Morphine	$7.7x10^{-7}$ M ($2.7x10^{-6}$-$8.7x10^{-7}$)
Distilled Water (4 days) + Morphine	$6.8x10^{-8}$ M ($3.2x10^{-8}$-$9.5x10^{-8}$).

Acute Opiate Dependence on Guinea-Pig Isolated Ileum

Concentration-Related Effect of DTγE on Morphine, DAGO, U50-488H, β-Endorphin and Deltorphin Withdrawal

DTγE ($1x10^{-9}$-$5x10^{-8}$-$1x10^{-8}$ M) administered 10 min before morphine, DAGO, U50-488H, β-endorphin or deltorphin induced a significant and concentration-dependent reduction on opiate withdrawal induced by μ, k and δ opioid agonists (Table **2**). DTγE IC_{50} was $5.3x10^{-8}$ M ($2.1x10^{-9}$-$3.4x10^{-8}$) for morphine, $4.4x10^{-8}$ M ($6.3x10^{-9}$-$8.9x10^{-8}$) for DAGO, $4.8x10^{-8}$ M ($2.3x10^{-9}$-$6.2x10^{-8}$) for U50-488H, $2.2x10^{-9}$ M ($1.2x10^{-9}$-$3.6x10^{-8}$) for β-endorphin, $2.8x10^{-9}$ M ($4.3x10^{-9}$-$2.7x10^{-8}$) for deltorphin. Very similar results were obtained when DTγE was injected after opioid agonists (Table **2**).

After washout, both acetylcholine response and electrical stimulation were not affected by DTγE treatment whereas the final morphine, DAGO, U50-488H, β-endorphin and deltorphin withdrawal control was still reduced (Table **3**).

Concentration-Related Effect of DEγE on Morphine, DAGO, U50-488H, β-Endorphin and Deltorphin Withdrawal

DEγE ($1x10^{-9}$-$5x10^{-8}$-$1x10^{-8}$ M) administered 10 min before morphine, DAGO, U50-488H, β-endorphin or deltorphin induced a significant and concentration-dependent reduction on opiate withdrawal induced by μ, k and δ opioid agonists (Table **2**). The $IC_{50\ of}$ DEγE was $2.1x10^{-8}$ M ($4.3x10^{-9}$-$5.7x10^{-8}$) for morphine, $2.3x10^{-8}$ M ($2.2x10^{-9}$-$1.7x10^{-8}$) for DAGO, $2.4x10^{-8}$ M ($4.8x10^{-9}$-$2.5x10^{-8}$) for U50-488H, $5.4x10^{-9}$ M ($1.0x10^{-9}$-$1.4x10^{-8}$) for β-endorphin, $5.3x10^{-9}$ M ($2.2x10^{-9}$-$1.8x10^{-8}$) for deltorphin. The same results were obtained when DEγE was injected after opioid agonists (Table **2**).

After washout, both acetylcholine response and electrical stimulation were not affected by DEγE treatment whereas the final morphine, DAGO, U50-488H, β-endorphin and deltorphin withdrawal control was still reduced (Table **3**).

DISCUSSION

Summary of the Findings

The results indicate that chronic treatment of guinea pigs with DTγE or DEγE induces a significant reduction of the inhibitory effect of morphine on the E.C. of guinea-pig ileum as well a reduction of opiate withdrawal *in vitro* thus confirming an important functional interaction between β-endorphin fragments and opioid system as previously reported [8].

Although the relationship between β-endorphin fragments and the opioid system has already been determined *in vivo* by using analgesic tests [8], this is the first paper which evaluates whether β-endorphin fragments interact directly or not with the opioid receptors by using the E.C. of guinea-pig ileum.

The results of our experiments indicate that, although DTγE and DEγE are not able to induce opioid-like effect, they are able to reduce significantly morphine effects on the guinea-pig ileum after chronic treatment thus confirming the absence of direct interaction with the opioid receptors [8]. Furthermore, the inability of DTγE and DEγE to block morphine effects after acute treatment may further support this hypothesis.

Possible Relationship Between β-Endorphin Fragments and Dopaminergic System In The Control of Morphine Effect

Although the possible mechanisms by which chronic treatment of guinea-pigs with DTγE and DEγE reduce the inhibitory effect of morphine on the E.C. as well as opioid withdrawal are still unclear, several possibilities should be considered.

A specific role for catecholamines in regulating morphine effects [25] has been reported. Dopaminergic system have been widely implicated in many of the pharmacological effects of opioids. Manipulations that alter the activity of dopamine frequenly modify the effects of morphine and other drugs [25]. Several studies have shown a link between DTγE, DEγE and monoamine system, most prominently re-

Table 2. ED50 and 95% C.I. Values of DTγE and DEγE ($1x10^{-9}$-$5x10^{-8}$-$1x10^{-8}$ M) on Morphine, DAGO, U50-488H, β-Endorphin or Deltorphin Withdrawal. DTγE and DEγE were Administered 10 min Before or After Each Opioid Agonist (O.A.)

Opiate Withdrawal	DTγE Before O.A.	DTγE After O.A.	DEγE Before O.A.	DEγE After O.A.
Morphine	$5.3x10^{-8}$ M ($2.1x10^{-9}$-$3.4x10^{-8}$)	$4.5x10^{-8}$ M ($3.7x10^{-9}$-$8.1x10^{-8}$)	$2.1x10^{-8}$ M ($4.3x10^{-9}$-$5.7x10^{-8}$)	$5.5x10^{-8}$ M ($2.6x10^{-9}$-$7.4x10^{-8}$)
DAGO	$4.4x10^{-8}$ M ($6.3x10^{-9}$-$8.9x10^{-8}$)	$5.7x10^{-8}$ M ($3.5x10^{-9}$-$7.2x10^{-8}$)	$2.3x10^{-8}$ M ($2.2x10^{-9}$-$1.7x10^{-8}$)	$4.2x10^{-8}$ M ($7.1x10^{-9}$-$2.7x10^{-8}$)
U50-488H	$4.8x10^{-8}$ M ($2.3x10^{-9}$-$6.2x10^{-8}$)	$2.9x10^{-8}$ M ($2.5x10^{-9}$-$3.4x10^{-8}$)	$2.4x10^{-8}$ M ($4.8x10^{-9}$-$2.5x10^{-8}$)	$3.7x10^{-8}$ M ($5.2x10^{-9}$-$7.4x10^{-8}$)
β-Endorphin	$2.2x10^{-9}$ M ($1.2x10^{-9}$-$3.6x10^{-8}$)	$5.8x10^{-8}$ M ($3.5x10^{-9}$-$5.2x10^{-7}$)	$5.4x10^{-9}$ M ($1.0x10^{-9}$-$1.4x10^{-8}$)	$6.2x10^{-8}$ M ($4.1x10^{-9}$-$8.2x10^{-8}$)
Deltorphin	$2.8x10^{-9}$ M ($4.3x10^{-9}$-$2.7x10^{-8}$)	$6.1x10^{-8}$ M ($6.7x10^{-9}$-$4.7x10^{-8}$)	$5.3x10^{-9}$ M ($2.2x10^{-9}$-$1.8x10^{-8}$)	$6.5x10^{-8}$ M ($1.4x10^{-9}$-$7.7x10^{-8}$)

garding the possible interference exerted by these β-endorphin fragments on the brain dopaminergic system [3]. Thus, it has been suggested that γ-type endorphins affect dopaminergic activity in the brain by modulating the self-inhibitory dopamine receptor system [26]. The results of the present study may support this suggestion, since it has been reported that the monoaminergic system is one of the several systems that can be implicated in the pharmacological effect of opioids [25]. In this regard, in our experimental model, DTγE and DEγE may interact with the monoaminergic system in the guinea-pig ileum reducing dopaminergic activity thus decreasing the inhibitory effect of morphine on the E.C. of guinea-pig ileum and opiate withdrawal. The results of the present study may support this suggestion, since it has been reported that the dopaminergic system is one of the several systems that can be implicated in the control of opioid withdrawal [27]. In this regard, in our experimental model, DTγE and DEγE may interact with dopaminergic system in the guinea-pig ileum reducing dopaminergic activity thus decreasing the inhibitory effect of morphine on the E.C. of guinea-pig ileum and opiate withdrawal.

Possible Indirect Modulation of Opioid System By DTγE and DEγE

Another possibility should be considered. Removal of the N-terminal residue tyrosine from γ-endorphin results in a loss of opioid activity, with a loss of the capacity of DTγE to interfere with opioid receptors [1]. On the other hand, the possibility that the effect of DTγE and DEγE on the inhibitory action of morphine on guinea-pig ileum and on the opioid withdrawal may be mediated through a modulation of opioid system activity (biosynthesis, storage or release of opioids) should be considered. This hypothesis may be indirectly supported by our recent data [8] showing that treatment with DTγE and DEgE reduce some morphine effects in a dose-dependent manner.

In conclusion, whatever the mechanism may be, the present study provides a further evidence that β-endorphin fragments influence morphine effect. The ability of DTγE and DEγE to reduce morphine effects *in vitro* supports the idea of a modulation of opioid system activity (biosynthesis,

Table 3. The Effect of of DTγE and DEγE ($1x10^{-9}$-$5x10^{-8}$-$1x10^{-8}$ M) on Ach Response, Electrical Stimulation (E.S.) and Final Opioid Withdrawal. DTγE and DEγE were Administered 10 min Before or after Each Opioid Agonist (O.A.)

Treatment	Ach Response	Electrical Stimulation	Final Opioid Withdrawal
DTγE Before O.A.	0	0	44.7±3.7**
DTγE After O.A.	0	0	49.1±2.7**
DEγE Before O.A.	0	0	57.3±3.5**
DEγE After O.A.	0	0	52.8±2.7**

Results are expressed as % of inhibition (mean±s.e.m.); *P<0.05; **P<0.01.

All data were compared to the pre-drug.

storage or release of opioids) or an involvement of the dopaminergic system.

ABBREVIATIONS

DEγE	=	Des-enkephalin-γ-endorphin
DTγE	=	Des-tyrosine-γ-endorphin
DAGO	=	D-Ala_2-N-methyl-Phe-Gly_5-ol)enkephalin
E.C.	=	Electrical stimulation
U50-488H	=	Trans(±)-3,4-dichloro-N-methyl-N-(2(1pyrrolidynyl)cyclohexyl)-benzeneacetamide

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Received: 22 October, 2008 Revised: 23 December, 2008 Accepted: 13 January, 2009