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β-Endorphin regulates diverse functions of splenic phagocytes through different opioid receptors in freshwater fish *Channa punctatus* (Bloch): An in vitro study

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Abstract
In this in vitro study, the role of β-endorphin in the control of phagocytic and cytotoxic activities of fish splenic phagocytes was investigated. Further, the involvement of specific opioid receptor was explored. β-Endorphin stimulated phagocytosis, whereas inhibited nitric oxide production as assessed by nitrite release. However, it had concentration-related biphasic effects on superoxide production, stimulatory at low and inhibitory at high concentration. Naltrexone, non-selective opioid receptor antagonist, antagonized the effect of β-endorphin on phagocyte functions. Moreover, CTAP, selective μ-receptor antagonist, completely blocked the effect of β-endorphin on phagocytosis and nitrite release. With regard to superoxide production, CTAP blocked the stimulatory effect of β-endorphin at low concentration, while the inhibitory effect at high concentration was completely antagonized by selective δ-receptor antagonist, NTI. In conclusion, β-endorphin acting via μ-receptor stimulated phagocytosis and inhibited nitric oxide production, while its biphasic effect on superoxide production seems to be mediated by μ- and δ-receptors.

1. Introduction

β-Endorphin (β-end) is a pro-opiomelanocortin-derived endogenous opioid peptide whose several immunoreactive forms are shown in fish pituitary gland [1–3]. *N*-acetylated form of β-end (NAc β-end) is predominantly released by melanotrophs in the pars intermedia and non-acetylated form mainly by corticotrophs in the pars distalis of the pituitary gland. The non-acetylated form of β-end is implicated in stress response since stress-associated corticotrophin-releasing hormone (CRH) stimulates the production of non-acetylated β-end from cells of the pars distalis in vitro [3]. On the other hand, inconsistent and diverse effects

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of CRH are seen on NAc β-end release from melanotrophs in the common carp, *Cyprinus carpio* [4].

Fish grown in intensive culture systems are frequently subjected to cognitive and non-cognitive stressors that induce the neuroendocrine system, and consequently, influence the immune system. The non-specific defense mechanism in teleost fish plays a crucial role especially when the specific immune responses are suppressed. It tends to counterbalance the specific immune responses at adverse environmental conditions until the specific immune system adapts [5]. Phagocytic cells, macrophages and neutrophils are the important constituents of non-specific immune system. They eliminate the pathogens through phagocytosis and by releasing cytotoxic and inflammatory substances. With regard to the direct role of β-end in the control of phagocyte activity, the report is limited in fishes [6]. Moreover, no report is available on the functional existence of opioid receptors on phagocytic cells in fishes. In view of this, the present study was undertaken to investigate the effect of non-acetylated form of β-end on phagocytic and cytotoxic activities of splenic phagocytes of spotted murrel *Channa punctatus*. Since an endogenous opioid ligand might interact with more than one type of opioid receptor, in the present study, the role of specific opioid receptor, μ (mu), δ (delta) or κ (kappa), in mediating the β-end effect on different functions of phagocyte was explored.

2. Materials and methods

2.1. Animals

The spotted snake-head *C. punctatus* belongs to a group of air-breathing fishes which by virtue of the presence of accessory respiratory organ, can thrive well in swampy derelict water bodies normally considered adverse for pisciculture due to low oxygen concentration. They survive for hours even under total dryness, whereas other air-breathing fishes like Clariids and Heteropneustids are vulnerable to complete desiccation. This group of fish constitutes the mainstay of pond fishery in the Indian subcontinent. The common availability, small size in comparison to other species in India such as giant murrel *Channa marulius* or stripped murrel *Channa striatus*, and least mortality while transportation, acclimation and experimentation led us to select the *C. punctatus* as the model for the experimental study.

Adult male fish weighing 80–100 g were procured from a neighboring state of Delhi (latitude 28.38° N, longitude 77.2° E). They were acclimated to the laboratory conditions for a fortnight under 12L:12D light regimens at 25 ± 2 °C, and fed with beef liver on alternate day. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Statistics and Programme Implementation, Government of India were followed for the maintenance and sacrifice of animals.

2.2. Reagents and culture medium

For the preparation of complete culture medium, 40 μg/ml gentamycin, 100 μg/ml streptomycin, 100 IU/ml penicillin (Ranbaxy, India), 5.94 mg/ml HEPES buffer and 10% heat-inactivated fetal calf serum (Biological Industries, Beth Haemek, Israel) were supplemented in cell culture medium RPMI-1640 (Sigma Chemicals, St. Louis, MO, USA). Naltrexone, phorbol 12-myristate 13-acetate (PMA) and nitro blue tetrazolium (NBT) were procured from Sigma Chemicals (St. Louis, MO, USA). Dimethyl sulphoxide (DMSO), 2-phenoxyethanol, methanol, Triton X-100, *N*-(1-naphthyl) ethylene diamine dihydrochloride (NEDD), sulphanilamide and other routine chemicals were purchased from Sisco Research Laboratories Pvt. Ltd./Merck Ltd. (India). Trypan blue was purchased from Central Drug House Pvt. Ltd. (India). MTT [3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide] was purchased from Amersham Biosciences (Sweden). Human β-end (NeoMPS, Inc., San Diego, CA, USA) was used in the present study since β-end of teleostean origin is not commercially available. The mammalian source of β-end is used in other fishes also [7–9]. Further, it is to be noted that the genes encoding β-end and its different receptors in fishes are highly conserved [10–14]. β-End and its different receptor antagonists, μ-receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2), $\delta_{1,2}$-receptor antagonist naltrindole (NTI) hydrochloride and κ-receptor antagonist norbinaltorphimine (NorBNI) dihydrochloride, were generously gifted by National Institute on Drug Abuse (Bethesda, USA).

2.3. Preparation of splenic phagocyte monolayer

Fishes were sacrificed by an overdose of 2-phenoxy ethanol (1:1000 v/v). Their spleens were dissected out and forced through a nylon mesh of 90 μm pore size into chilled phosphate-buffered saline (PBS, pH 7.8). The cell suspension was centrifuged at 600*g* for 15 min. The supernatant was discarded. Red blood corpuscles were lysed by hypotonic shock treatment following the method of Paltrinieri et al. [15] with minor modifications. Cell suspension was centrifuged, washed and resuspended in the complete culture medium. The cell suspension was flooded on pre-washed slides (200 μl/slide) or added to 96-well (100 μl/well) culture plate. Phagocytes were allowed to adhere for 2 h. The non-adherent cells were washed off with PBS. The density of phagocytic cells was $\sim 1 \times 10^6$ cells/ml. All the experiments were carried out in an incubator maintained at 25 °C (±0.1) with 5% CO_2.

2.4. In vitro experiments

2.4.1. Dose-related effect of β-endorphin

Splenic phagocytes were incubated with different concentrations of β-end ranging from 10^{-13} to 10^{-5} M for 90 min. The duration of treatment was determined based on time-kinetics of β-end in our pilot experiments. Cells cultured without hormone were considered as the control. After incubation, phagocytes were washed with PBS and used for phagocytosis, super oxide production and nitrite release.

2.4.2. Effect of non-selective opioid receptor antagonist, naltrexone

Based on dose-related effect, the phagocyte monolayer was incubated with 10^{-9} M β-end and 10^{-7} M naltrexone, simultaneously, for phagocytosis and nitrite release. In case

of superoxide production, two different sets of experiments depending on concentration-related dual effects were conducted: (i) 10^{-13} M β-end+10^{-11} M naltrexone, (ii) 10^{-5} M β-end+10^{-4} M naltrexone, simultaneously. To compare the results, phagocytes were incubated with β-end/naltrexone alone. As a separate control, cells were cultured in medium alone. After 90 min, cells were washed and used for phagocytosis, superoxide and nitrite production.

2.4.3. Effect of selective antagonist for μ, δ and κ-receptor

To understand the functional existence of specific opioid receptors, the phagocyte monolayer was incubated simultaneously with β-end and its selective μ-/δ-/κ-receptor antagonists, CTAP, NTI, NorBNI, respectively, for 90 min. For phagocytosis and nitrite release, they were incubated with 10^{-9} M β-end and 10^{-7} M of CTAP/NTI/NorBNI, while 10^{-13}/10^{-5} M β-end and 10^{-11}/10^{-4} M of CTAP/NTI/NorBNI were used for superoxide production. As controls, cells were also incubated in medium alone, with the respective concentration of β-end, or its different selective antagonists. After incubation, phagocytes were washed and used for phagocytosis, nitrite and superoxide production.

2.5. Phagocytic assay

For phagocytic assay, splenic phagocytes were incubated with heat-killed yeast cells for 90 min, washed with PBS, fixed in methanol and stained with giemsa. Without any predetermined sequence or scheme, approximately 200 phagocytes/slide were counted. The technical details on each slide were covered prior to counting. Therefore, the experimenter was not aware of any technical details of slide while counting. Phagocytes that engulfed one or more than one yeast cells are counted as positive cells. The percentage of phagocytosis and the phagocytic index were calculated following the formulae: (a) percentage phagocytosis—number of phagocytic cells showing phagocytosis per 100 phagocytic cells observed; (b) phagocytic index—average number of engulfed yeast cells per positive cell $\times$ percentage of phagocytosis.

2.6. Superoxide assay

The intracellular superoxide anion was determined by the reduction of redox dye NBT following the method of Sakai et al. [16] with slight modifications. The phagocytic cells were incubated in medium containing 1 mg/ml NBT and 1 μg/ml PMA for 2 h in 96-well culture plate. Cells were washed and fixed with methanol. The reduced intracellular product, formazon, was dissolved by adding 20 μl of 0.1% Triton X-100, 120 μl of 2 M KOH and 140 μl DMSO. The absorbance was measured at 620 nm by a multiscan spectrophotometer.

2.7. Nitrite assay

The monolayer was incubated in complete medium for 48 h. The cell-free supernatant was collected and processed for nitrite assay following the method of Ding et al. [17] with minor modifications. In brief, the phagocyte-conditioned medium was mixed with an equal volume of Griess reagent (1% sulphanilamide, 0.1% NEDD and 2.5% H_3PO_4). After 10 min of incubation at room temperature, the absorbance was measured at 540 nm using an ELISA plate reader. The standard curve of sodium nitrite was used to calculate the amount of nitrite in the conditioned medium. The concentration of nitrite is expressed as micromolar (μM).

2.8. Cell viability

The effect of different concentrations of β-end on cell viability of phagocytes was assessed by trypan blue exclusion test and MTT assay.

2.8.1. Trypan blue exclusion test

After treatment with β-end for 90 min, cells were washed and stained with 0.4% trypan blue. Approximately 200 phagocytes/slide were counted without any predetermined sequence or scheme. The percentage of live cells that excluded the dye due to intact plasma membrane was calculated.

2.8.2. MTT assay

The MTT assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of MTT and form dark blue formazan crystals which are largely impermeable to the cell membrane. Solubilization of cells by the addition of a detergent results in the liberation of the crystals. The number of surviving cells is directly proportional to the level of the formazan product created. The assay was performed using the method of Mosmann [18] with minor modifications. After treatment with different concentrations of β-end for 90 min, cells were washed and incubated in 100 μl of the medium containing 0.5 mg/ml MTT for 2 h in 96-well culture plate. The phagocytes were washed and solubilized by adding 150 μl of DMSO. After 15 min incubation at room temperature, the absorbance was measured at 570 nm by a multiscan spectrophotometer.

2.9. Statistical analysis

Each set of experiment was carried out in triplicate and repeated thrice with cells collected from different animals to verify the reproducibility of the results. The data of one of the repeated experiments were analysed by one-way analysis of variance (ANOVA) followed by Newman Keuls' multiple range test, and presented as mean $\pm$ SEM.

3. Results

3.1. Dose-related effect of β-endorphin

β-End showed dose-related significant variation (ANOVA, $P<0.001$) in phagocytosis, superoxide and nitric oxide production. β-End markedly increased the phagocytic activity of splenic phagocytes at 10^{-13} M as compared with control (Newman Keuls' multiple range test, $P<0.01$). The β-end-induced increase of phagocytosis reached its maximum at 10^{-9} M. With the further increase

of concentrations, the phagocytic activity sharply decreased. However, the percentage of phagocytosis and phagocytic index at 10^{-7} or 10^{-5} M β-end were still significantly higher than that incubated in medium alone ($10^{-7}/10^{-5}$ M β-end vs. control, $P<0.01$, Fig. 1A). On the contrary to phagocytosis, β-end significantly ($P<0.01$) inhibited the nitric oxide production at 10^{-13} M and the inhibition increased considerably with the increase of its concentration up to 10^{-9} M (Fig. 1B, 10^{-13} vs. 10^{-9} M, $P<0.01$). However, further increase in the β-end concentration resulted in the marked decrease of its inhibitory effect on nitric oxide production (10^{-9} vs. 10^{-5} M, $P<0.01$). In case of superoxide production, β-end had concentration-dependent biphasic effects. The maximum stimulation of superoxide production was observed at 10^{-13} M. The β-end-induced stimulation decreased with the increase of its concentration and culminated into marked inhibition at 10^{-5} M (10^{-5} M β-end vs. medium alone, $P<0.01$, Fig. 1C).

β-End at any of its concentrations failed to affect the cell viability as determined by trypan blue exclusion test. The percentage viability of β-end-treated phagocytes was comparable to those incubated in medium alone (Fig. 2A). Similar results were observed following MTT assay (Fig. 2B).

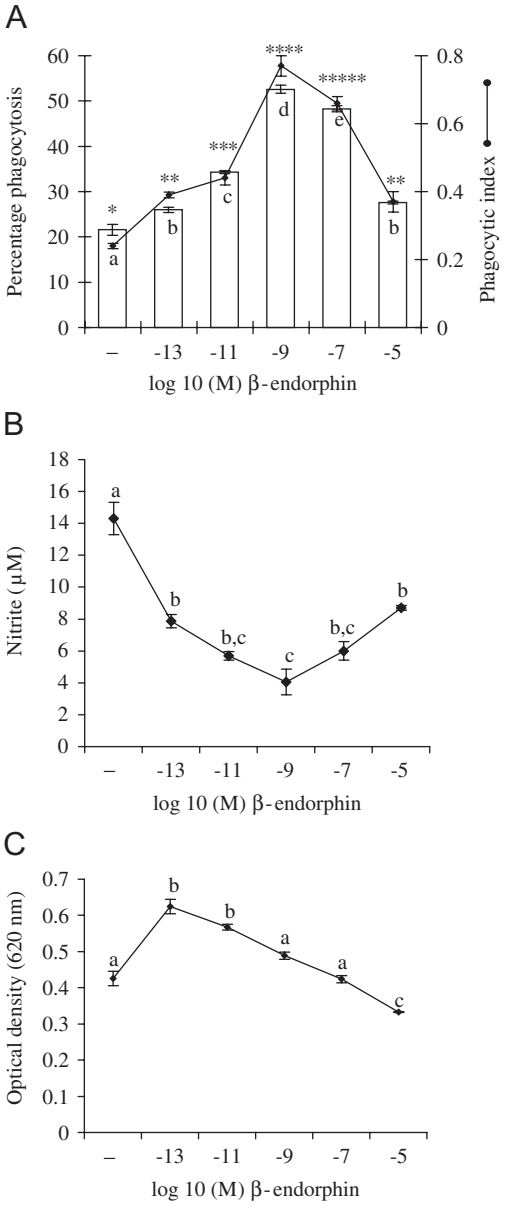

Fig. 1 In vitro effect of β-endorphin on the percentage of phagocytosis and phagocytic index (A) bar graph and line graph, respectively, nitrite release (B) and superoxide production (C) by splenic phagocytes. Phagocytes incubated in medium alone were considered as control (−). Each sample was run in triplicate and the experiment was repeated three times ($n=3$) with different fishes to verify the reproducibility of results. For each experiment, spleens of three fishes were pooled to prepare the phagocyte monolayer. Data (mean ± SEM) represent one of the independent experiments. Error bars bearing different superscripts differ significantly (Newman Keuls' multiple range test, $P<0.01$).

3.2. Effect of non-selective opioid receptor antagonist, naltrexone

Naltrexone antagonized the stimulatory effect of β-end on phagocytosis when splenic phagocytes were incubated with

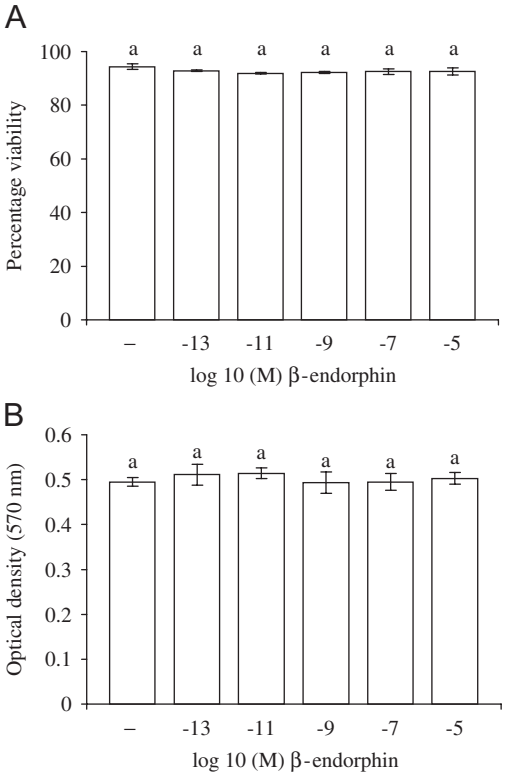

Fig. 2 Dose-related effect of β-endorphin on viability of splenic phagocytes following trypan blue exclusion test (A) and MTT assay (B). Each sample was run in triplicate. The experiment was repeated three times ($n=3$) with different fishes. Data (mean ± SEM) represent one of the independent experiments. The error bars bearing same superscripts do not differ significantly (Newman Keuls' multiple range test, $P<0.05$).

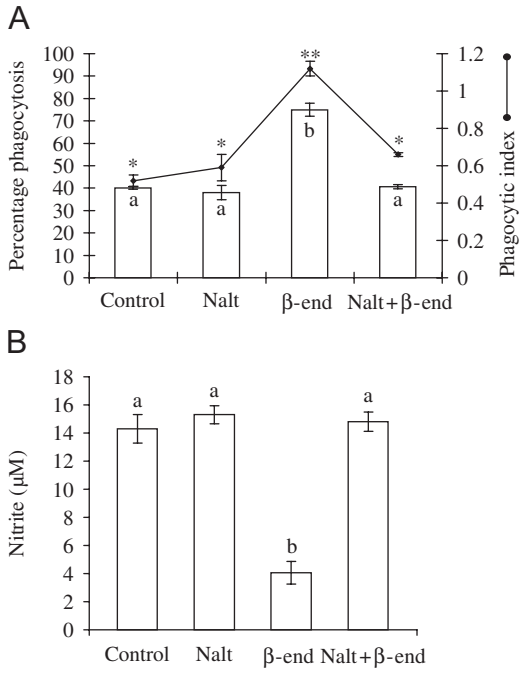

Fig. 3 Effect of non-selective opioid receptors antagonist, naltrexone (Nalt, 10^{-7} M) on β-endorphin (β-end, 10^{-9} M)-induced stimulation of phagocytosis (A), and inhibition of nitrite release (B) by splenic phagocytes. Each sample was run in triplicate, and repeated three times ($n = 3$) with different fishes. Data (mean $\pm$ SEM) represent one of the independent experiments. The error bars bearing different superscripts differ significantly (Newman Keuls' multiple range test, $P < 0.01$).

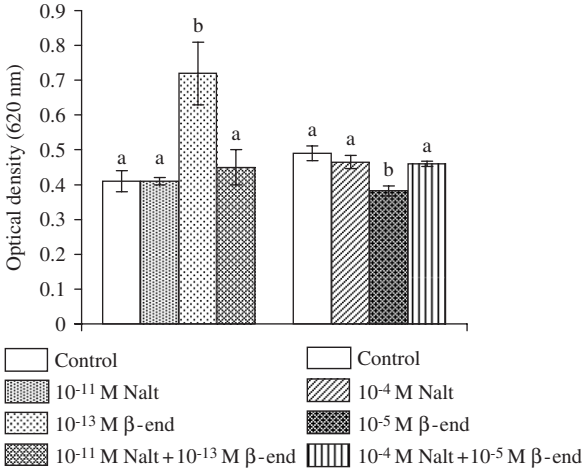

Fig. 4 Effect of naltrexone on concentration-dependent β-endorphin-induced stimulation and inhibition of superoxide production. In the case of stimulation, phagocytes were incubated simultaneously with 10^{-13} M β-endorphin and 10^{-11} M naltrexone, while 10^{-4} M naltrexone and 10^{-5} M β-endorphin were used simultaneously to observe the antagonistic effect of non-selective opioid receptor blocker on β-endorphin-inhibited superoxide production. Each sample was run in triplicate. The experiment was repeated three times ($n = 3$) with different fishes. Data (mean $\pm$ SEM) represent one of the independent experiments. The error bars bearing different superscripts differ significantly (Newman Keuls' multiple range test, $P < 0.01$).

β-end and naltrexone, simultaneously (Fig. 3A). Similarly, it completely blocked the inhibitory effect of β-end on nitric oxide production (Fig. 3B) or concentration-dependent biphasic effects on superoxide production (Fig. 4). However, naltrexone alone did not influence the phagocyte activities at any of its concentration.

3.3. Effect of selective opioid receptor antagonists

Splenic phagocytes incubated with β-end and its selective μ-, δ- and κ- receptor antagonists showed the involvement of different opioid receptors in mediating the effect of β-end on different functions of phagocytes. Selective μ-receptor antagonist CTAP, and not the δ- and κ-receptor antagonists, completely antagonized the effect of β-end (β-end+CTAP vs. β-end, $P < 0.01$) on phagocytosis (Fig. 5A) and nitric oxide production (Fig. 5B). In case of superoxide production, the concentration-dependent biphasic effects of β-end were blocked by different opioid receptor antagonists. The stimulatory effect of β-end at 10^{-13} M was totally blocked by selective μ-receptor antagonist CTAP (Fig. 6A), while the inhibitory effect at 10^{-5} M was fully antagonized by δ-receptor antagonist, NTI (Fig. 6B). However, κ-receptor antagonist, NorBNI failed to modulate the effect of β-end on any function of phagocytes.

4. Discussion

The present in vitro study in *C. punctatus* showed the stimulatory effect of β-end on phagocytic activity of splenic phagocytes at all the tested concentrations. This is consistent with the in vitro reports in rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio* where β-end is shown to stimulate the phagocytic activity of phagocytic cells from head kidney [6]. Similar response is observed in in vivo study with β-end in rainbow trout [19]. Although basal level of β-end in blood plasma is not estimated in spotted murrel *C. punctatus*, approximately 10^{-9} M concentration of β-end is reported in trout [20] and common carp [3] plasma. Together, it appears that in fishes β-end enhances the phagocytosis under normal physiological condition. Interestingly, in the present study, the maximum stimulation at 10^{-9} M gradually decreased with the increase in concentrations. It is to be noted that β-end did not affect the cell viability of splenic phagocytes even at high concentrations. This reflects that the decline in phagocytosis at high concentration of β-end in the present study was not due to the cell death. Rather, it might be due to the desensitization of receptors, since the magnitude of desensitization of opiate receptors is shown to be dependent on the concentration and exposure time of opioid agonist [21].

Although study on the functional existence of opioid receptors with regard to phagocytosis is lacking in fishes, both opioid receptor-dependent and -independent mechanisms for β-end actions are shown in mammals [22]. In the

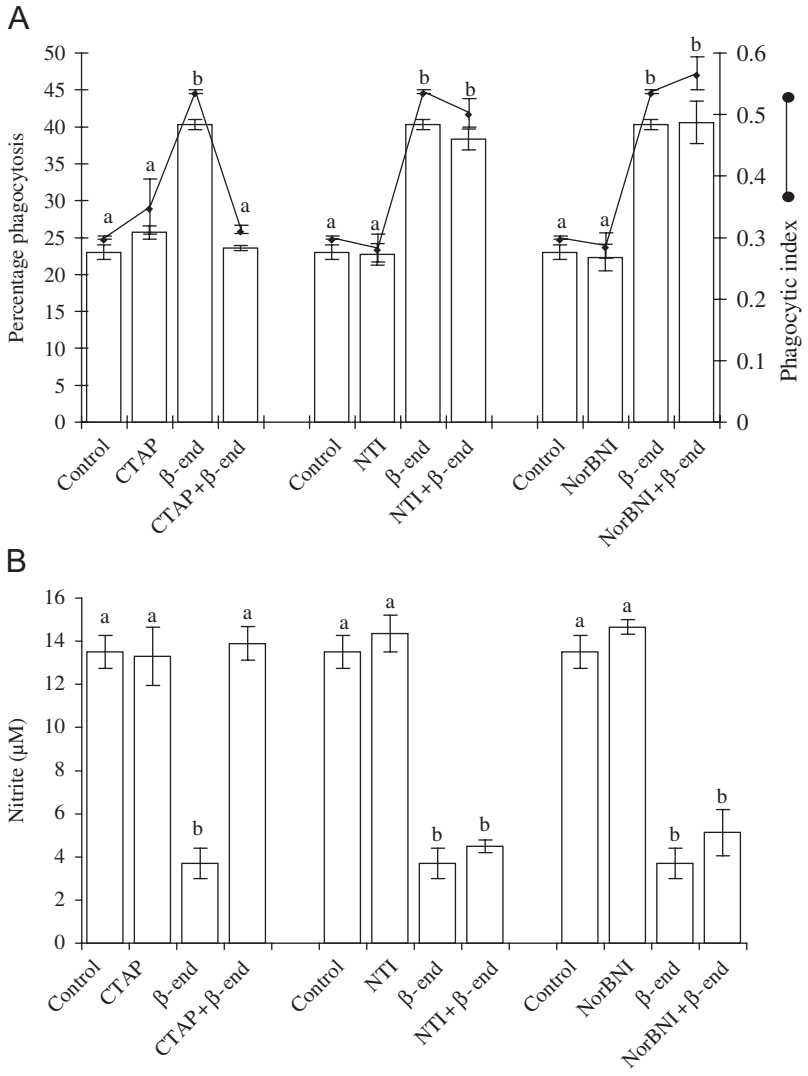

Fig. 5 Effect of selective μ-receptor antagonist, CTAP, selective δ-receptor antagonist, NTI, and selective κ-receptor antagonist, NorBNI on β-endorphin (10^{-9}M)-induced phagocytosis (A) and nitrite release (B) by splenic phagocytes. Antagonists were used at hundred times higher concentration than β-end. Each sample was run in triplicate, and repeated three times ($n = 3$) with different fishes. Data (mean $\pm$ SEM) represent one of the independent experiments. The error bars bearing different superscripts differ significantly (Newman Keuls' multiple range test, $P<0.01$).

present study, non-selective opioid receptor antagonist, naltrexone, completely antagonized the stimulatory effect of β-end on phagocytosis, and thereby, suggesting the involvement of opioid receptors in mediating the stimulatory effect of β-end on phagocytic activity of splenic phagocytes. Further, using selective opioid receptor (μ, δ and κ) antagonists, the exclusive involvement of μ-receptor was shown in mediating the effect of β-end on phagocytosis in spotted murrel. This is in parallel to the report in mice where μ-receptor agonist, and not the δ or κ agonist, has been reported to affect the phagocytic activity of peritoneal macrophages [23]. Surprisingly, opioid receptor-independent mechanism is shown to be responsible for β-end effect on phagocytosis by peritoneal macrophages in mouse [24], and the difference in specificity of target cells (e.g. *Candida albicans*/*Escherichia coli* or latex particle) to be phagocytosed is speculated for this discrepancy in β-end action.

The role of β-end in the regulation of nitric oxide production by phagocytes is probed for the first time in fishes. The effect of β-end on nitrite release by splenic phagocytes of spotted murrel was in contrast to that observed on phagocytosis. β-End had inhibitory effect on nitrite release. However, like phagocytosis, the exclusive involvement of μ-receptor was seen in mediating the effect of β-end on nitrite production. Similar observations are reported in mammals. β-End is shown to decrease the PMA-induced nitrite release by rat alveolar macrophages [25]. Further, μ-receptor-mediated inhibition of nitrite production by murine macrophage cell line, J774, has been demonstrated using selective μ-receptor agonist DAGO [26].

In addition, the present study elucidates the role of β-end in the control of respiratory burst activity of splenic phagocytes as assessed by its effect on PMA-induced superoxide production. β-End had concentration-related

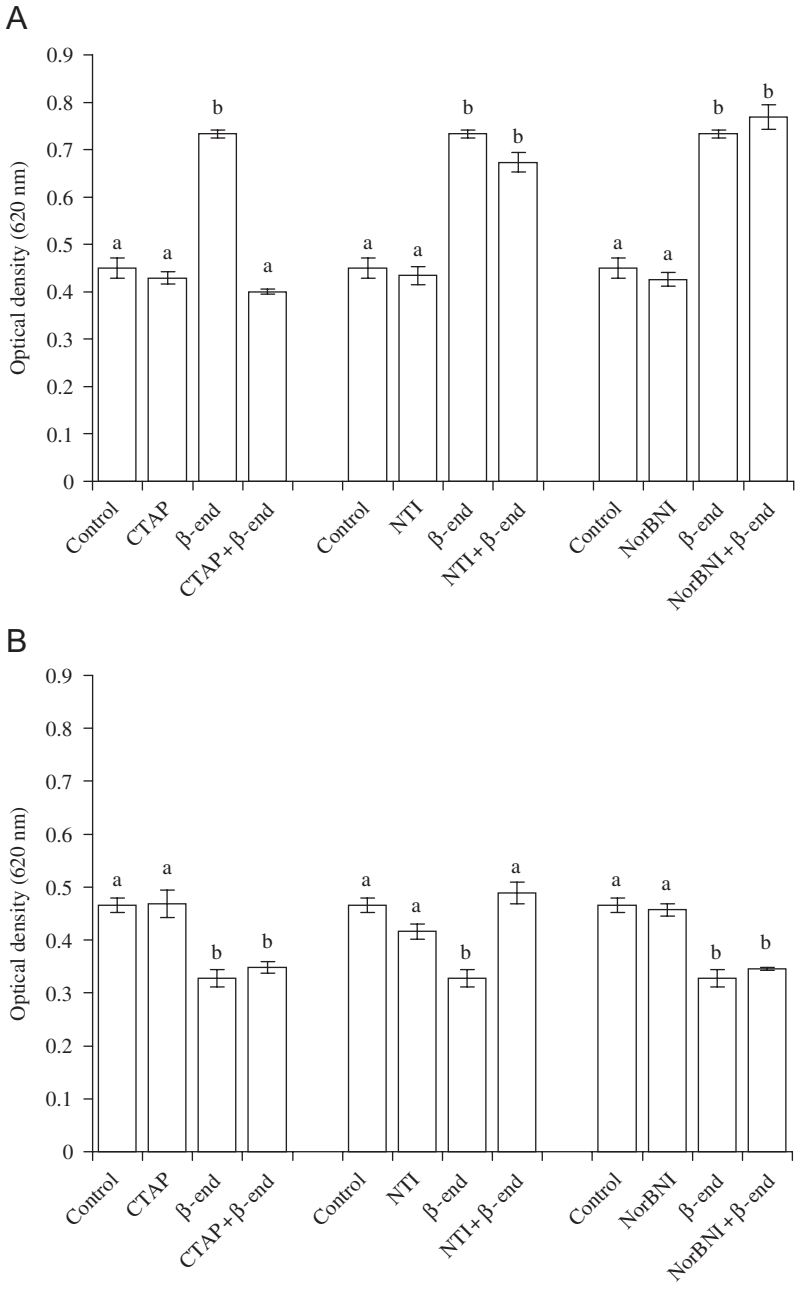

Fig. 6 Effect of selective opioid receptor antagonist, μ (CTAP), δ (NTI) and κ (NorBNI) on concentration-dependent β-endorphin-induced stimulation (A) and inhibition (B) of superoxide production. In case of stimulation, phagocytes were incubated with 10^{-13} M β-endorphin and 10^{-11} M CTAP/NTI/NorBNI, simultaneously. 10^{-5} M β-endorphin and 10^{-4} M CTAP/NTI/NorBNI were used to observe the antagonistic effect of selective opioid receptor blocker on β-endorphin-induced inhibition of superoxide production. Each sample was run in triplicate. The experiment was repeated three times ($n = 3$) with different fishes. Data (mean $\pm$ SEM) represent one of the independent experiments. The error bars bearing different superscripts differ significantly (Newman Keuls' multiple range test, $P<0.01$).

differential effects, stimulatory at low concentrations, inhibitory at high concentration and no effect at concentrations ranging from 10^{-9} to 10^{-7} M. These results in spotted murrel are in contrast to the reports in rainbow trout and common carp where β-end is shown to stimulate the superoxide production by kidney phagocytic cells at all concentrations ranging from 1 to 100 ng/ml [6]. The in vivo study in the rainbow trout also reports the dose-dependent stimulatory effect of β-end on superoxide production by kidney leukocytes [19]. Moreover, in rainbow trout naloxone partially antagonized the effect of β-end on superoxide production by kidney phagocytic cells [6], implicating the involvement of some other mechanism along with opioid receptors in mediating the effect of β-end on respiratory burst activity. However, the concentration-dependent biphasic effects of β-end on superoxide production by splenic

phagocytes of spotted murrel was completely abrogated by naltrexone, implicating the sole involvement of opioid receptors in β-end-regulated superoxide production in *C. punctatus*. Further, the involvement of different opioid receptors, μ and δ, in mediating the stimulatory and inhibitory effect, respectively, of β-end on superoxide production was demonstrated in the present study. Although the role of μ-receptor in regulating superoxide production by human monocytic cell line THP-1 has been recently reported using selective high-affinity μ-opioid receptor agonists, endorphins 1 and 2 [27], the involvement of δ in modulating β-end effect on respiratory burst activity is not shown so far. To develop a conceptual understanding, molecular characterization of opioid receptors and their density on phagocytic cells need to be investigated in different groups of vertebrates.

The earlier study using radioligand binding technique in goldfish *Carassius auratus* reports the presence of two different categories of opioid receptors on leukocytes [28]. One of them shows the characteristics similar to those of μ_3 receptors, while other resembles in its affinity with the κ receptors. In addition, the density of the κ receptors on goldfish leukocytes is reported to be considerably higher than μ_3 receptors [28]. The understanding on the presence of different types of opioid receptors was further developed by cloning and molecular characterization of opioid receptor genes from other immune cells of zebrafish. The three important genes encoding different opioid receptors are cloned and characterized from zebrafish *Danio rerio* and referred as ZFOR1 [12,29], ZFOR2 [13] and ZFOR3 [14]. Also, a full-length cDNA for a μ-receptor (CCMOR) and partial clones for δ and κ receptors are cloned from another teleost fish *Catastomus commersoni* [11]. Further, ZFOR1, ZFOR2/CCMOR and ZFOR3 are shown to share a high degree of homology with the counter opioid receptor, δ, μ and κ, respectively, in mammals. These observations indirectly support our speculation on the existence of different functional opioid receptors on phagocytic cells in spotted murrel using selective antagonists. Also, the pharmacological profile of specific opioid receptor in *C. punctatus* seems to be similar to that of mammals.

Nevertheless, the present findings suggest that β-end regulates different phagocyte functions in fish *C. punctatus* by acting through different opioid receptors. It stimulates phagocytosis and inhibits nitric oxide production acting through the μ-receptor, whereas it enhances superoxide production at lower concentrations via μ-receptor and decreases the respiratory burst activity at higher concentrations via δ-receptor. This complex multi-receptor interaction of β-endorphin helps in maintaining the immune homeostasis during physiological conditions and in stressful situations that might help in the better survival of fishes.

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