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Full Length Research Paper

Melatonin Modulation of Air-Gulping Behavior in the Freshwater Fish *Channa punctatus* (Bloch)

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ABSTRACT:

Air-gulping behavior is of considerable significance in those species that have evolved and perfected air-breathing organs. Data available on details of air-breathing behavior is Indian fishes is scarce. Therefore, this study was undertaken to gather information on temporal organization pineal control air-gulping behavior in *Channa punctatus.* Pineal hormone melatonin is known to regulate biological rhythms and is therefore regarded as a chronobiotic. For this reason the effect of exogenously administered melatonin on air-gulping rhythm was assessed. The frequency of air-gulping increased ($P<0.01$) at 10.00h and 16.00h, the maximum increase at 16.00h. The observation from second experiment confirmed that air-gulping frequency is high ($P<0.01$) at 10.00h and 16.00h; and melatonin further increases ($P<0.01$) air-gulping at the same time points. Melatonin given at the evening point was significantly ($P<0.01$) more effective in increasing the air-gulping frequency. Using or not using aerator did not significantly affect the rhythm in air-gulping behavior. Dissolved Oxygen content remains unchanged before and after the experiment. In all the experiments air-gulping behavior exhibited a definite rhythm. In *Channa punctatus* the frequency of air-gulping coincided with scotophase (late in the light phase). Could it be that melatonin heralds the onset of scotophase and thereby enhance air-gulping. Alternately, melatonin may enhance metabolism at tissue level increasing the oxygen demand, which in turn could enhance air-gulping activity.

Key words: Melatonin, Air-gulping frequency, Dissolved Oxygen, Aerator

INTRODUCTION

Channa punctatus (Bloch) is the most common species of murrel which constitute a dominant group of air-breathing fishes in India. Air-gulping behavior is of considerable significance in those species that have evolved and perfected air-breathing organs. Data available on details of air-breathing behavior is Indian fishes is scarce. Therefore, this study was undertaken to gather information on temporal organization pineal control air-gulping behavior in *Channa punctatus.* Pineal hormone melatonin is known to regulate biological rhythms and is therefore regarded as a rhythm hormone or a chronobiotic. For this reason the effect of exogenously administered melatonin on air-gulping rhythm was assessed.

MATERIAL AND METHODS

The teleost fish *Channa punctatus* (Bloch) were collected from Bheema river (N latitude 17^0) and kept for acclimatization to laboratory conditions in glass aquaria for two weeks prior to their use in the experiments. During this period they were treated with antibiotic chloramphenicol (5 mg/l of water), as prophylactic agent.

Three experiments were conducted. For all the experiments the fish were held in aquaria (23" x 46") with 14 liters of water. During the course of experiment the animals were fed with live earthworm on alternate days and the water of the aquaria changed daily. The water temperature was maintained at 21 ± 1^0 C. Air-gulping behavior was observed visually 10 minutes after the treatment and the observation was made for 15 minutes for all the experiments. In 15 minutes of duration, the number of times the fish surfaced to water to gulp air was recorded for all the fish.

First experiment was conducted to observe the rhythm of air-gulping behavior in this species. Frequency of air-gulping was recorded in control (ethanol-saline treated) and melatonin (10µg intra muscularly) treated animals at 10:00, 12:00, 14:00 and 16:00hrs respectively. On the basis of the result observed in the first experiment that air-gulping frequency was more at 10.00h and 16.00hrs only, the second experiment was undertaken. In which frequency of air-gulping was recorded at 10:00h and 16:00h in control (ethanol-saline treated) and melatonin (10µg) treated groups respectively containing two fish in each group. The third experiment was carried out to observe the effect of melatonin and rhythms using aerator (aerator was used to test whether the observed effects were due to changes in dissolved oxygen content or not. Therefore Dissolved Oxygen (D.O) was calculated before and after the injection for both vehicle and melatonin injected groups) using Wrinkler's Iodometric method. In this experiment sixteen fishes were divided into eight groups of two animals each. I - IV groups were held in aquarium without aerator. Among which two groups received ethanol-saline treatment (Control) at 10:00h and 16:00h and other two groups received Melatonin treated at 10:00hand 16:00h. The remaining groups (V-VIII) were held in aquarium with aerator. The treatment for these groups was similar to those of group I-IV. Dissolved Oxygen was calculated using Wrinkler's iodometric method. Statistical analysis was done by using a computer program for ANOVA and Schiff's Pairwise Comparison test.

RESULTS

The observation from the first experiment result reveals that the teleost fish *Channa punctatus* has rhythm in air-gulping behavior. The frequency of air-gulping is increased ($P<0.01$) at 10.00h and 16.00h when compared to the frequency at 12.00h and 14.00h groups, the maximum increase was observed at 16.00h (Figure-1). Treatment with melatonin further increased the frequency of air gulping at 10.00, 16.00hrs (Figure-1). The observation from second experiment only confirmed that air-gulping frequency is high ($P<0.01$) at 10.00h and 16.00h; and melatonin further increases ($P<0.01$) the number surfaces visits of the fish to gulp air at the same time points. Melatonin given at the evening point was significantly ($P<0.01$) more effective in increasing the air-gulping frequency (Figure-2). In the third experiment the air-gulping frequency of fish held in aquaria that were fitted with aerator were compared to the frequency of air-gulping in fish that were held in aquaria without aerator. The results were similar to those observed in first and second experiment. Using or not using aerator did not significantly affect the rhythm in air-gulping behavior. Dissolved oxygen content remains unchanged before and after the experiment (Figure-2).

DISCUSSION

Deficiency of dissolved oxygen in water bodies could have been responsible for the evolution of air- breathing behavior in fish. Air-breathing behavior in a particular species of fish may be development commensurate with its habitat. Air-breathing in fish is either obligatory or facultative. *Channa punctatus* has been regarded by earlier workers as a facultative air-breather (Hakim et la., 1983). However, in this study *Channa punctatus* revealed rhythmic behavior in air-breathing irrespective of whether an aerator was used or not.

In all the experiments air-gulping behavior exhibited a definite rhythm. Air-gulping frequency increased at 10.00h followed by decrease at 12.00 and 14.00h and reaching peak at 19.00h. However to have a complete understanding of the rhythms in air-gulping behavior it is necessary to measure the frequency round the clock using automated measuring devices. In Heteropneustes fossilis a circadian rhythm in air-gulping activity was reported (Maheshwari, 1998). In this fish the frequency of air-gulping coincided with the onset of scotophase (late in the light phase).

Air-gulping behavior in *Channa punctatus* did not seem to depend on oxygen tension. Titrimetric measurement of dissolved oxygen was slightly higher at 16.00h; logically one would have expected a decrease in the frequency of air-gulping. On the contrary the air-gulping frequency peaked at this hour indicating that the built-in rhythmic behavior perhaps is independent of oxygen tension of the water. Only more investigations would clarify if *Channa punctatus* could be classified as an obligatory air-breather.

Treatment with melatonin did not alter the rhythmic behaviors; it only augmented frequency of air-gulping activity at both 10.00h and 16.00h. It is established that hormones do influence the organization of behavior (Alcock, 2001). Pineal gland is perhaps not involved in the entrainment of air-gulping activity in the Indian walking catfish *Clarias batrachus* (Yadu and Shedpure, 2002).

In the present study however, melatonin did increase the he frequency of air-gulping even though the rhythm as such was not disturbed. Another study indicated the importance of diel cycle in synchronization of locomotor and air-breathing activity in *Hoplosternum littorale* (Boujard et al., 2006). Since changes in diel cycle are known to affect pineal production of melatonin (Reiter et al., 1986), it is logical to believe that pineal may have an important role in regulation of air-gulping behavior too. It is well known that melatonin is arhythm hormone and is involved in the regulation of most physiological rhythms (Reiter, 1984). Further studies will be needed to elucidate the role of pineal and its hormone melatonin in the regulation air-gulping behavior.

The mechanism by which melatonin enhanced the frequency of air-gulping is not yet clear. Melatonin possibly heralds the onset of scotophase and thereby enhances air-gulping. Alternately, melatonin may enhance metabolism at tissue level increasing the oxygen demand which in turn could enhance air-gulping activity.

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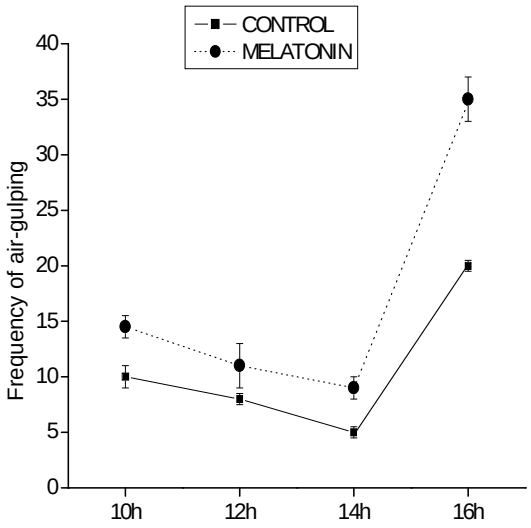
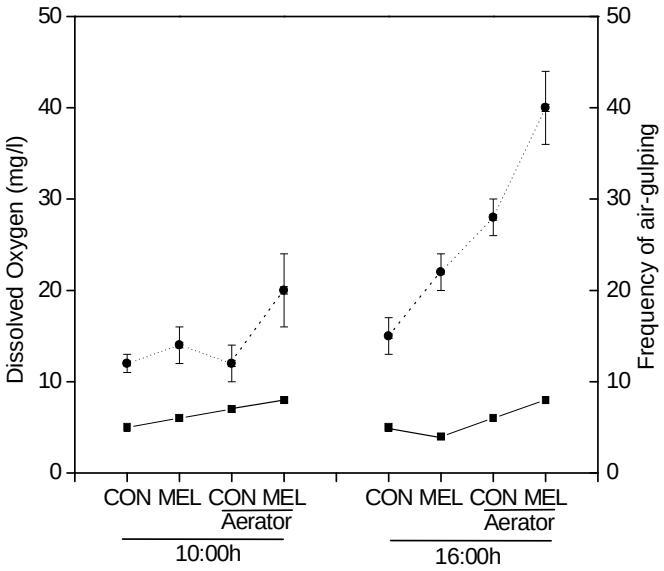
Figures

(1)


CONTROL
MELATONIN
Frequency of air-gulping
0
5
10
15
20
25
30
35
40
10h
12h
14h
16h

(2)


Dissolved Oxygen (mg/l)
Frequency of air-gulping
0
10
20
30
40
50
CON MEL CON MEL
Aerator
10:00h
CON MEL CON MEL
Aerator
16:00h