

Morphometrical studies of reproductive system of birds after treatment with dopamine receptor blockers and melatonin

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Received: 7 October 2005; accepted: 10 October 2006

SUMMARY

Male Japanese quails were treated with melatonin alone or melatonin combined with D1 and D2 dopamine receptor blockers. Following the treatment, hypothalamus, pituitary glands and testes were analyzed morphometrically. The results suggest the existence of an interaction between melatonin and dopaminergic system in the brain in the regulation of reproductive processes in immature birds. The character of this interaction alters according to the time of the treatment (morning, afternoon, evening, night). *Reproductive Biology* 2006, 6, Suppl. 2:87–92.

Key words: reproductive system, melatonin, dopamine receptors, birds

INTRODUCTION

Several lines of evidence indicate that some effects of melatonin on the reproductive axis in mammals [2, 3, 4] and fish [1] are mediated by the

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dopaminergic system of the brain. In animals, dopamine action requires dopamine receptors (DR), mainly D₁R and D₂R. Blocking the receptors prevents dopamine from affecting its target tissues. The response of reproductive tissues to melatonin in association to the involvement of dopamine was not investigated in birds.

The aim of this study was to determine if melatonin affects the reproductive axis in birds by means of the dopaminergic system. Moreover, we tested if such action may be affected by the time of day. To address this issue, we treated male Japanese quails, on four different hours, with dopamine receptor blockers, and then with melatonin. Since functional activity of the reproductive tract is accompanied by respective morphological changes, morphometrical analysis of selected parameters associated with reproduction (diameter of nuclei of neurons in hypothalamic arcuate nucleus, cross-sectional area of nuclei of gonadotrophs, diameter of seminiferous tubules in the testis) was performed in immature birds.

MATERIALS AND METHODS

The experiment was carried out on 160 five-weeks-old male Japanese quails (*Coturnix japonica*) (bw 125±10 g) kept under 14L:10D lighting schedule (light on at 7:00 am) and fed with a standard mixture *ad libitum*. The birds were treated: a/ im with dopamine D₂ receptor (D₂R) blocker-haloperidol (30 µg/100 g bw; Gedeon Richter, Hungary) or saline (SAL; 0.2 ml/bird); b/ icv with dopamine D₁ receptor (D₁R) blocker - R(+)-SCH-23390 (8 µg/100 g bw; Sigma, USA) or SAL (2 µl/bird); c/ orally with melatonin (MEL; 10 µg/100 g bw; Genzyme Corporation, USA) or placebo (P). Twenty-eight treatment groups (n=5-6 birds per group) were generated and housed in separate cages. One block of treatments included: SAL-, MEL-, D₂R blocker-, D₁R blocker-, D₂R blocker+MEL-, D₁R blocker+MEL-, and D₁R blocker+D₂R blocker+MEL-treated group. The block of treatments was repeated four times: in the morning, in the afternoon, in the evening, and at night. The detailed treatment schedule is depicted in Table 1.

Table 1. Treatment schedule of Japanese quails administered with dopamine receptor blockers (D_1R and D_2R) and melatonin (MEL)

Group number →		1	2	3	4	5	6	7
Way of administration	Treatment time	8	9	10	11	12	13	14
		15	16	17	18	19	20	21
		22	23	24	25	26	27	28
im	7 am 1 pm 7 pm 1 am	SAL	SAL	D_2R blocker	D_2R blocker	SAL	SAL	D_2R blocker
icv	7 am 1 pm 7 pm 1 am	SAL	SAL	SAL	SAL	D_1R blocker	D_1R blocker	D_1R blocker
orally	8 am 2 pm 8 pm 2 am	P	MEL	P	MEL	P	MEL	MEL
Sacrificed respectively at 10 am 4 pm 10 pm 2 am								

SAL: saline; MEL: melatonin; P: placebo; im: intramuscular injection; icv: intracerebroventricular infusion into a third ventricle of a brain

Intracerebroventricular infusion of R(+)-SCH-23390 was performed using stereotaxis instrument SEZH-3 ("Medexport" with chicken beak adaptor) by syringe into the third ventricle of the brain. The quails were anesthetized with 5% ketamine (Biolik, 0.1 ml/100 g bw, im; Ukraine) before icv infusion of R(+)-SCH-23390 or SAL. Before administration, 30 μ g of haloperidol was dissolved in 0.2 ml of saline and 8 μ g of R(+)-SCH-23390 was dissolved in 2 μ l of saline. Melatonin was administered orally as a tablet. Birds were decapitated two hours after the last treatment. During night hours, examined tissues were collected under red dim light.

The hypothalamic area of the brain, pituitary and testes were dissected *post mortem* from each bird, fixed in Bouin's fixative, dehydrated, and embedded in paraffin. Serial sections (5 μm) of the hypothalamic area were cut in a sagittal plane and stained with cresyl violet. Testicular sections (5 μm) were stained with haematoxylin-eosin. In each experimental group, diameters of 150 cellular nuclei of neurons in arcuate nucleus (D-AN) and diameters of 150 seminiferous tubules (D-ST) from each experimental group were measured using micrometrical ocular MOB-1-15x. The pituitaries were cut (5 μm) and stained with alcian blue, Schiff's reagent and orange G. Gonadotrophs appeared as red. For morphometric comparison, 150 cross-sectional areas of nucleus of gonadotrophs (S-Gon) from each experimental group were measured using computer software Promorph-Paradise for Windows. All data are presented as means \pm SEM. Statistical analysis of data was performed using Student's t-test (STATISTICA 5.0 for Windows; StatSoft Inc., Tulsa, OK, USA). Values were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Melatonin treatment performed in the morning did not affect (tab. 2) the diameter of nuclei of neurons in arcuate nucleus (D-AN). In the afternoon, however, melatonin decreased, and in the evening and at night increased this diameter. In the presence of D_2R blocker, melatonin increased the D-AN at almost all examined times; the only exception was the evening melatonin treatment when no effect of D_2R blocker was found. In the presence of D_1R blocker, melatonin decreased the D-AN in the morning and evening but did not affect the examined parameter following the afternoon or night treatment. Double blockade of DR always decreased the D-AN in comparison with melatonin treatment alone.

The cross-sectional area of nuclei of gonadotrophs (S-Gon) did not change after melatonin treatment in the morning, but decreased in the presence of melatonin and DR blockers. Moreover, morning melatonin treatment decreased the diameter of seminiferous tubules (D-ST), and increased the D-ST in the presence of both DR blockers. A similar effect for the D-ST was observed after introduction of melatonin and D_1R blocker in the

Table 2. Morphometrical data (means±SEM) of hypothalamo-hypophyseal-gonadal axis in Japanese quails treated with dopamine receptor blockers (D₁R and D₂R) and melatonin (MEL)

# Group	Experimental group	Diameter of nuclei of neurons in arcuate nucleus (μm)	Cross-sectional area of nuclei of gonadotrophs (μm ²)	Diameter of seminiferous tubules (μm)
Sacrificed at 10:00 am (morning)				
1	SAL	7.7±0.1	10.2±0.3	84±3
2	MEL	7.9±0.2	9.1±0.4	53±1*
3	D ₂ R blocker	7.8±0.1	8.6±0.2	79±2
4	D ₂ R blocker+MEL	9.1±0.1*^	8.0±0.2*^	107±4*^
5	D ₁ R blocker	6.7±0.1*	8.1±0.2*	76±2*
6	D ₁ R blocker+MEL	7.2±0.1*^	8.3±0.1*^	76±2*^
7	D ₁ R blocker+D ₂ R blocker+MEL	6.6±0.1^	8.5±0.2*^	95±3*^
Sacrificed at 4:00 pm (afternoon)				
8	SAL	7.9±0.1	9.1±0.3	96±2
9	MEL	7.4±0.1*	10.7±0.1*	130±7*
10	D ₂ R blocker	7.7±0.1	7.6±0.1*	102±2
11	D ₂ R blocker+MEL	9.8±0.2*^	8.9±0.3^	64±2*^
12	D ₁ R blocker	7.1±0.1*	7.9±0.2*	88±3
13	D ₁ R blocker+MEL	7.4±0.1*	8.8±0.1^	77±2*^
14	D ₁ R blocker+D ₂ R blocker+MEL	6.9±0.1^	8.8±0.3^	93±3^
Sacrificed at 10:00 pm (evening)				
15	SAL	7.8±0.1	9.3±0.2	109±5
16	MEL	8.8±0.1*	9.5±0.3	85±4*
17	D ₂ R blocker	8.2±0.1*	7.9±0.2*	97±2*
18	D ₂ R blocker+MEL	9.1±0.2*	8.7±0.2*	92±3*
19	D ₁ R blocker	7.6±0.1	8.8±0.2*	82±2*
20	D ₁ R blocker+MEL	7.0±0.1*^	9.4±0.1	160±4*^
21	D ₁ R blocker+D ₂ R blocker+MEL	6.9±0.1^	9.1±0.1	114±3^
Sacrificed at 4:00 am (night)				
22	SAL	7.2±0.1	9.4±0.3	108±2
23	MEL	7.6±0.1*	10.2±0.3*	97±4*
24	D ₂ R blocker	8.6±0.2*	8.2±0.2*	88±3*
25	D ₂ R blocker+MEL	9.2±0.1*^	8.8±0.3^	84±5*^
26	D ₁ R blocker	7.1±0.1	9.1±0.1	77±1*
27	D ₁ R blocker+MEL	7.4±0.1	8.1±0.1*^	87±2*^
28	D ₁ R blocker+D ₂ R blocker+MEL	6.9±0.1^	7.9±0.2*^	131±4*^

*indicates significant difference (p<0.05) in comparison to the corresponding parameter in the saline (SAL) group; ^indicates significant difference (p<0.05) in comparison to the corresponding parameter in the melatonin (MEL) group

evening. D₂R blocker administration in the evening did not influence the melatonin effect on the D-ST. No melatonin effect on the S-Gon was found in the evening. Melatonin increased the S-Gon and D-ST administered in the afternoon. At night melatonin increased the S-Gon and decreased the D-ST. Treatment with D₂R and/or D₁R blocker in the afternoon decreased the stimulatory effect of melatonin on the S-Gon and D-ST. A similar effect has been observed for the S-Gon at night. At this time, each of the DR blockers decreased the inhibitory effect of melatonin on the D-ST. On the contrary, the double blockade of DR increased the D-ST at night.

In most cases, combined treatments of melatonin and DR blockers had converse consequences in comparison with melatonin treatments alone e.g. morning treatment of melatonin alone decreased D-ST, and treatment of melatonin and D₂R blocker increased D-ST. In addition, the D-AN and

S-Gon were not changed after morning treatment of melatonin, but D-AN increased and S-Gon decreased after treatment with melatonin and D₂R blocker. Moreover, the evening treatment with melatonin as well as with D₁R blocker decreased D-ST, but treatment with melatonin and D₁R blocker increased D-ST. These differences in examined parameters (and others presented in the Table 2) after melatonin treatment and melatonin plus DR blockers suggest the existence of an interaction between melatonin and dopaminergic system in the brain in the regulation of reproductive processes in immature birds. The character of this interaction alters according to the time of the treatment (morning, afternoon, evening, night). More detailed investigation is necessary to clarify the character of such interaction.

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