Differential effects of histamine on Leydig cell and testicular macrophage activities in wall lizards: precise role of H1/H2 receptor subtypes

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Abstract

The present study in the wall lizard, *Hemidactylus flaviviridis*, was aimed to understand the role of histamine (HA) in the regulation of Leydig cell and testicular macrophage activities, for the first time, in ectothermic vertebrates. Although HA did not affect the testosterone production from unstimulated Leydig cells, it had dual concentration-related effects, stimulatory at a low concentration of 10^{-10} M while inhibitory at a high concentration of 10^{-5} M, on FSH-induced testosterone production. This suggests that HA did not influence the basal Leydig cell steroidogenesis, but modulated the FSH-stimulated testosterone production in a biphasic manner depending upon its concentration. However, HA failed to affect the FSH-stimulated Leydig cell proliferation, indicating that HA modulated the testosterone production from Leydig cells

without influencing their proliferation in wall lizards. HA, apart from Leydig cells, differentially regulated the testicular macrophage immune responses. It inhibited phagocytosis and superoxide production at high concentration (10^{-5} M), while stimulated superoxide production and could not affect phagocytosis at low concentration (10^{-10} M). Using selective H1 and H2 antagonists, pyrilamine and famotidine respectively, H1 receptor subtype was seen responsible for mediating the inhibitory effect of HA on Leydig cell steroidogenesis and testicular macrophage immune responses at high concentration, while H2 receptors were involved for the stimulation at low concentration.

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Introduction

Research on paracrine control of Leydig cell activity in mammals has primarily focused upon the factors secreted from Sertoli cells in the seminiferous tubule (Lejeune *et al.* 1996, Grisswold & Mclean 2006). In the last decade, there has been a groundswell of interest in understanding the interrelationship between Leydig cells and testicular macrophages (Haider 2004, Hutson 2006). Moreover, lymphocytes and low number of mast cells are also present in the interstitial compartment of the testis (Gaytan *et al.* 1989, Pinart *et al.* 2001). Mast cells are shown to be the main source of histamine (HA) in the testis (Albrecht *et al.* 2005). In addition, germ cells are also reported to add in testicular HA milieu (Safina *et al.* 2002). In recent years, efforts have been made to investigate the role of HA in control of Leydig cell steroidogenesis, though the reports are limited and the results are contradictory (Mayerhofer *et al.* 1989, Mondillo *et al.* 2005). Intriguingly, the role of HA in control of testicular macrophage immune responses has not been studied so far, despite the fact that mast cells and macrophages are present in the testicular microenvironment and known for their immunoregulatory functions (Beer *et al.* 1984, Azuma *et al.* 2001, Fijak & Meinhardt 2006, Hedger & Hales 2006).

In wall lizards, testicular macrophages are present in substantial numbers along with Leydig cells in a ratio of 1:3–4 in the interstitial tissue (unpublished observation). The presence of mast cells in testicular interstitial tissue has been demonstrated in the lizard *Podarcis s. sicula* (Minucci *et al.* 1995) and other non-mammalian vertebrate (Di Matteo *et al.* 2000). However, no information is available on the role of HA, principally a mast cell-secreted product, in paracrine regulation of Leydig cell and macrophage activities in ectothermic vertebrates. In the light of these observations, the present study in wall lizards was aimed to provide direct evidence of the role of HA in homeostatic control of Leydig cell steroidogenesis and proliferation, and testicular macrophage immune responses. In addition, attempts were also made to demonstrate the existence of specific functional HA receptors on Leydig cells and testicular macrophages.

Materials and Methods

Animals

Adult male wall lizards, *Hemidactylus flaviviridis*, of 8–10 g body weight were procured locally (Delhi: latitude,

28°12'–28°53' N; longitude, 76°50'–77°23') in the months of May to July (regressed phase) and acclimated to laboratory conditions for 1 week prior to the experiment. They were maintained (12 h light:12 h darkness) in wooden cages with wire mesh on the sides and top, and fed live insects *ad libitum*. 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 in the maintenance and killing of animals.

Reagents and culture media

Dulbecco's modified Eagle's medium/Hams F-12 medium (DMEM/F-12, 1:1 mixture), collagenase type 1, bacterial lipopolysaccharide (*Escherichia coli* Serotype 55:B5), phorbol myristate acetate (PMA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), nitroblue tetrazolium (NBT), percoll, SDS, HA dihydrochloride, and selective antagonists pyrilamine (P) and famotidine (F) for HA receptor subtypes H1 and H2 respectively were purchased from Sigma chemicals Co. Ovine follicle-stimulating hormone (oFSH-16, AFP-5592C) was a kind gift from NIDDK (Torrance, CA, USA). As per the technical details of oFSH, the contamination with other pituitary hormones was negligible. Giemsa stain was obtained from Merck India Ltd and other routine chemicals were purchased from SRL Pvt Ltd (Mumbai, India). Tritiated thymidine (methyl-³H-TdR; 2.0 Ci/mmol) was purchased from the Board of Radiation and Isotope Technology (BRIT, Bombay, India).

The culture medium was supplemented with 40 µg/ml gentamycin, 100 µg/ml streptomycin, 100 IU/ml penicillin (Ranbaxy India Ltd, New Delhi, India), 50 µM β-mercaptoethanol, and 25 mM HEPES buffer (Merck India Ltd), and the pH was adjusted to 7.2. The fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel) was heat inactivated and added to the culture medium prior to use.

Isolation of testicular interstitial cells

Leydig cell-enriched fraction Leydig cells from regressed testes of wall lizards were isolated following the procedure of Khan & Rai (2005). In brief, the lizards were killed, and the testes were taken out, pooled, and transferred to cold DMEM/F-12 medium containing 2% FCS. After washing, the testes were decapsulated, chopped, resuspended in cold culture medium, and shaken gently to disperse the tubules. The suspension was kept on ice for 15–20 min at unit gravity to sediment the tubules. The supernatant containing Leydig cells, macrophages, and other interstitial cells were decanted. This process was repeated thrice. The pellet of interstitial cells was obtained following centrifugation at 200 *g* for 5 min at 4 °C (step 1). In addition, to minimize the loss of Leydig cells, a pellet of seminiferous tubules was treated with collagenase (0.9 mg/ml), shaken in a water bath for 10–15 min at 25 °C,

1 ml FCS was added, and the suspension was kept on ice to stop the enzymatic reaction. The dispersed tubular suspension was passed through the tea strainer to remove the tubules. After that, the suspension was centrifuged at 200 *g* to obtain the Leydig cells (step 2). The pellet of Leydig cells was washed thrice. Finally, the Leydig cell pellets from step 1 and 2 were resuspended in 1 ml culture medium for percoll gradient centrifugation. The maximum number of Leydig cells present in 30–40% of percoll gradient were separated out by centrifugation at 200 *g* for 5 min at 4 °C. The pellet of Leydig cells was washed and resuspended in culture medium containing 5% FCS. The density of Leydig cells was adjusted to 1.6×10^6 cells/ml. They were identified by $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase enzyme test. The viability was determined by Trypan blue exclusion. The purity of Leydig cells ranged from 80 to 90% with 70–75% viability. Prior to any experiment, Leydig cells were incubated in medium alone for 24 h at 25 °C (± 0.1) with 5% CO₂.

For testosterone assay, 1 ml/well of Leydig cell suspension was added to a 24-well culture plate. After treatments, the testosterone was estimated in the Leydig cell-conditioned medium by radioimmuno assay (RIA) (Abraham 1974). For Leydig cell proliferation following calorimetric MTT assay (Tian & Song 2006) or [³H]TdR incorporation (Khan *et al.* 1992) methods, 200 µl/well of Leydig cell suspension was added to a 96-well culture plate. In the case of the MTT assay, after the treatments, the Leydig cells were washed and incubated with 200 µl MTT working soluble (1 mg/ml) for 2 h. Thereafter, cells were washed and solubilized with acidic isopropanol (0.5% SDS, 0.04 M HCl in 90% isopropanol) for 1 h. The absorbance was measured at 570 nm. For [³H]TdR incorporation assay, the Leydig cells were pulsed with 1 µCi [³H]TdR/well 5 h prior to the completion of incubation. After culture, the Leydig cells were harvested by centrifugation, washed with PBS, and lysed with 1% SDS (100 µl/well). The radioactivity of the lysate was counted in a liquid scintillation counter (Beckman Fullerton, CA, USA) and the proliferation was expressed in terms of c.p.m./ 3×10^5 cells.

Macrophage-enriched fraction

Macrophages were separated out from testicular interstitial cell suspension following the procedure of Mayerhofer *et al.* (1992) with minor modification. In brief, 200 µl interstitial cell suspension (1.6×10^6 cells/ml) was flooded on a prewashed slide or added to a 96-well culture plate (200 µl/slide or per well). The macrophages were allowed to adhere by incubating at 25 °C in a CO₂ incubator for 1 h. Non-adherent interstitial cells were washed off with PBS. In the adherent cell population, more than 90% of cells were macrophages ($\sim 0.4 \times 10^6$ cells/ml), as determined by their morphology following phase contrast, light, and electron microscopy and non-specific esterase activity. Prior to the experiment, macrophages were cultured in medium alone for 24 h. After treatments, phagocytic and respiratory burst

activities were examined to assess the testicular macrophage immune responses.

The phagocytic assay was performed following the method of Mondal & Rai (1999). Each slide with adhered testicular macrophages was flooded with a 200 μ l heat-killed yeast cell suspension (4×10^7 cell/ml). After 90 min, the monolayer was washed with PBS, fixed in methanol, and stained with Giemsa. Approximately 100 macrophages on each slide were observed for phagocytosis without any predetermined sequence. The percentage of phagocytosis and the phagocytic index were calculated using the formulae described by Campbell *et al.* (2001): a) percentage of phagocytosis = number of cells showing phagocytosis/100 macrophages, and b) phagocytic index = percentage of phagocytosis $\times$ number of yeast cells engulfed by a single macrophage. Prior to counting, the technical details of the treatment on each slide were covered. Therefore, the experimenter was blind to the technical details of the slides while counting.

For respiratory burst activity, the intracellular superoxide anion in testicular macrophages was determined by the reduction of redox dye (NBT) following the procedure of Sakai *et al.* (1996). After treatments, testicular macrophage monolayer was incubated with PBS containing 1 mg/ml NBT and 1 μ g/ml PMA for 2 h, washed, and fixed in methanol. The reduced intracellular product, formazon, was dissolved in 20 μ l of 0.1% triton X-100, 120 μ l of 2 M KOH, and 140 μ l dimethyl sulfoxide (DMSO). The absorbance was recorded at 620 nm by multiscan spectrophotometer.

In vitro experiments

Effect of HA and HA antagonists on Leydig cell activities To understand the role of HA in control of Leydig cell activities during the inactive and active states, *in vitro* concentration-related effect of HA was studied on non-stimulated and FSH-stimulated Leydig cells collected from regressed testis. It would be of interest to note that mammalian FSH is shown to regulate both the testicular functions in many squamates including wall lizards (Callard & Ho 1980, Khan & Rai 2004, 2005). In addition, cDNA for only FSH-receptor is cloned so far in squamates (Borrelli *et al.* 2001, Bluhm *et al.* 2004). Therefore, in the present study, FSH was used to stimulate the Leydig cell steroidogenesis and proliferation.

Forty-two lizards were killed for each non-stimulated and FSH-stimulated experimental group. The Leydig cells were isolated and incubated with different concentrations of HA from 10^{-10} to 10^{-5} M, in the presence or the absence of 0.5 μ g/ml FSH (FSH-stimulated and non-stimulated groups) for 24 h. To compare the results, Leydig cells were also incubated in the medium alone or with FSH for 24 h. Thereafter, the conditioned media were processed for testosterone assay and the Leydig cells for proliferation assay.

Selective H1 and H2 receptor antagonists, P and F respectively, were tested for their ability to antagonize the effect

of HA on testosterone production by FSH-stimulated Leydig cells. Concentrations of H1 and H2 antagonists ten times higher than HA were used, and therefore, two sets of experiments were made depending on low and high concentration of HA (10^{-5} and 10^{-10} M) used therein. Thirty-six lizards were killed to isolate the Leydig cells for each set of experiments. In the presence of FSH (0.5 μ g/ml), the Leydig cells were treated as follows: i) 10^{-5} M HA + 10^{-4} M P/F and ii) 10^{-10} M HA + 10^{-9} M P/F. As regards their respective controls, the Leydig cells were incubated with FSH alone, FSH + 10^{-5} / 10^{-10} M HA, and FSH + 10^{-4} / 10^{-9} M P or F. After 24-h treatment, Leydig cell-conditioned media were processed for testosterone assay.

Effect of HA and HA antagonists on testicular macrophage immune responses

Thirty-six lizards were killed to prepare the testicular macrophage monolayer for each HA or HA-antagonist experiment. The macrophages were incubated with different concentrations of HA (10^{-10} – 10^{-5} M) for 3 h. The duration of treatment was finalized based on the literature for rats (Azuma *et al.* 2001) and our pilot experiment in wall lizards. To assess the effect of H1 and H2 antagonists, P and F respectively, on HA-induced macrophage immune responses, testicular macrophages were incubated with high and low concentrations of HA and HA antagonists as follows: i) 10^{-5} M HA + 10^{-4} M P/F, ii) 10^{-10} M HA + 10^{-9} M P/F, iii) 10^{-5} M HA, iv) 10^{-10} M HA, v) 10^{-4} M P/F, and vi) 10^{-9} M P/F for 3 h. After incubation, the macrophage monolayer was washed and processed for phagocytic and superoxide assay.

Statistical analysis

Each treatment was carried out in triplicates and repeated thrice ($n=3$) with different animals to get the reproducibility of results. The data of a single independent experiment were analyzed by one-way ANOVA, compared by Newman–Keuls multiple range test, and represented as mean $\pm$ S.E.M. One-way ANOVA on ranks was especially applied in case of concentration-related effects of HA on testosterone production by FSH-stimulated Leydig cells and superoxide production by testicular macrophages.

Results

Effect of HA and HA antagonists on Leydig cell activities

Although HA did not influence the testosterone production from non-stimulated Leydig cells, it had dose-related biphasic effects on FSH-stimulated Leydig cell steroidogenesis (Fig. 1a). The FSH-stimulated testosterone production significantly ($P<0.01$) increased at a concentration of 10^{-10} M HA. The additive effect of HA, however,

Figure 1 Graph showing the role of histamine (HA) and its specific receptors (H1 and H2) in regulation of Leydig cell steroidogenesis. (a) Non-stimulated and FSH-stimulated Leydig cells were incubated with varying concentrations of HA ranging from 10^{-10} to 10^{-5} M. The testosterone was assayed in the condition medium. (b₁ and b₂) Effect of selective H1 and H2 receptor antagonists, pyrilamine (P) and famotidine (F) respectively, on HA-induced differential modulation, stimulation at 10^{-10} M (b₁), and inhibition at 10^{-5} M (b₂), of FSH-stimulated testosterone production. Each treatment was carried out in triplicates. Data (mean $\pm$ s.e.m.) represent one of the three independent experiments ($N=3$). At each time, different lizards were used. Different letters above the error bars indicate that the groups differ significantly at least at $P<0.05$.

decreased with the increase of its concentration. Rather, it inhibited the FSH-induced testosterone production at concentrations higher than 10^{-8} M in a dose-dependent manner (Fig. 1a). Further, the additive effect of 10^{-10} M HA on FSH-stimulated testosterone production was completely antagonized when Leydig cells were incubated with H2 antagonist, F, while the same was unaffected by the H1 antagonist, P (Fig. 1b₁). On the contrary, the inhibitory effect of 10^{-5} M HA on FSH-stimulated testosterone production by Leydig cells was antagonized by P only (Fig. 1b₂). Moreover, no detectable change in testosterone level was observed for P or F alone at a concentration of 10^{-9} / 10^{-4} M (Fig. 1b₁ and b₂).

Unlike steroidogenesis, MTT assay (Fig. 2a) or [3 H]TdR incorporation (Fig. 2b) exhibited that HA had no effect even on FSH-stimulated Leydig cell proliferation.

Effect of HA and HA antagonists on testicular macrophage immune responses

HA significantly ($P<0.01$) inhibited the phagocytic activities of testicular macrophages at 10^{-7} M, while concentrations lower than 10^{-7} M were ineffective as compared with the control. Moreover, a marked ($P<0.01$) increase in HA-induced suppression was observed with the increase of its concentration from 10^{-7} to 10^{-6} M. However, the inhibition of phagocytic activity at 10^{-5} M was comparable with that observed at 10^{-6} M (Fig. 3a). With regard to involvement of HA receptors, the inhibitory effect of HA (10^{-5} M) on the percentage of phagocytosis and the phagocytic index was totally antagonized by H1 antagonist, P. The H2 antagonist, F, did not influence the effect of HA on phagocytosis (Fig. 3b).

Figure 2 Graph showing no effect of HA on FSH-stimulated Leydig cell proliferation following (a) MTT assay and (b) [³H]TdR incorporation assay. Data expressed as mean $\pm$ s.e.m. are the representative of one of the repeated independent experiments ($n=3$) using different lizards.

On superoxide production, HA had concentration-dependent biphasic effects, maximally stimulatory at 10^{-10} M and inhibitory at 10^{-5} M. The HA-induced stimulation significantly decreased at 10^{-9} M (10^{-10} vs 10^{-9} M, $P<0.01$), and culminated in marked inhibition at 10^{-8} M as compared with the control ($P<0.05$). Further, HA-induced inhibition increased with the increase of its concentration from 10^{-8} to 10^{-5} M in a dose-dependent manner (Fig. 4a). Both H1 and H2 antagonists selectively antagonized the concentration-dependent biphasic effects of HA on superoxide production (Fig. 4b₁ and b₂). The H2 antagonist, F, blocked the stimulatory effect of HA at low (10^{-10} M) concentration (Fig. 4b₁), whereas P nullified the HA-induced inhibition of superoxide production at high (10^{-5} M) concentration (Fig. 4b₂). Moreover, P or F alone did not have any effect on macrophage immune responses at a concentration of $10^{-9}/10^{-4}$ M (Fig. 4b₁ and b₂).

Figure 3 Graph showing the role of histamine (HA) and its specific receptors (H1 and H2) in regulation of testicular macrophage phagocytosis. (a) Concentration-related effect of HA on the percentage of phagocytosis and the phagocytic index. (b) Effect of H1 and H2 receptor antagonists, P and F respectively, on 10^{-5} M HA-induced inhibition of the percentage of phagocytosis and the phagocytic index of testicular macrophages. Each treatment was carried out in triplicate, and repeated thrice ($N=3$) using different animals. Data of one of the representative experiments are shown as mean $\pm$ s.e.m. Values bearing different superscripts differ significantly at $P<0.01$.

Discussion

In the present study, oFSH was used to stimulate the Leydig cell activity since mammalian FSH is reported to control spermatogenesis as well as steroidogenesis in most of the lizards and snakes (Callard & Ho 1980, Rai & Haider 1986, Khan & Rai 2004, 2005), while luteinizing hormone (LH) was seen either ineffective or less potent in stimulating any of the testicular functions (Licht *et al.* 1977, Callard & Ho 1980, Rai & Haider 1986). In addition, high-affinity binding sites for mammalian FSH are demonstrated in intratubular and interstitial elements of lizard *Cnemidophorus* testis (Licht & Midgley 1977). Moreover, only cDNAs encoding FSH-receptor have been described so far in the gonads of lizard (Borrelli *et al.* 2001) and snake (Bluhm *et al.* 2004), and attempts to clone cDNA closely related to LH receptor were unsuccessful (Bluhm *et al.* 2004). With regard to the existence

Figure 4 Graph showing the role of HA and its specific receptors in regulation of superoxide production by testicular macrophage. (a) *In vitro* concentration-related biphasic effects of HA on superoxide production. Macrophages incubated in medium alone were considered as control. (b₁ and b₂) Effect of H1 and H2 receptor antagonists, P and F respectively, on HA-induced stimulation at 10⁻¹⁰ M (b₁) and inhibition at 10⁻⁵ M (b₂) of superoxide production by testicular macrophages. To compare the results, testicular macrophages were incubated with 10⁻¹⁰/10⁻⁵ M HA or 10⁻⁹/10⁻⁴ M P or F alone. Data of one of the independent experiments that are repeated thrice (*n*=3) using different animals are expressed as mean ± S.E.M. Values bearing different superscripts differ significantly at least at *P*<0.05.

of pituitary gonadotropins, unlike mammals and many non-mammalian vertebrates, including chelonians and crocodilians of reptiles (Licht 1984, Suzuki *et al.* 1988, Ando & Ishi 1994, Saito *et al.* 2002, Shen & Yu 2002), biochemical and molecular biology studies identify single gonadotropin in squamates (Licht *et al.* 1977, 1979, Aizawa & Ishi 2003). In wall lizards, an *in vitro* experiment was performed, where oFSH significantly enhanced the steroidogenesis and proliferation of Leydig cells (Khan & Rai 2005), whereas oLH had no marked effect (unpublished observation). The reported lack of FSH/LH specificity and the presence of single gonadotropin receptor (FSH receptor) may be attributed to single gonadotropin controlling all the testicular functions in most of the squamates (lizards and snakes). This may also be seen in light of reports where porcine FSH receptors recognize turtle LH better than turtle FSH (Moyle *et al.* 1994), and chicken LH is shown to bind with rat FSH receptor at an affinity higher than that of chicken FSH (Miya *et al.* 1994).

The present study showed the differential effects of HA on testosterone production depending upon its concentration and the state of Leydig cells. HA could increase or decrease the testosterone production at low or high concentration respectively, only when Leydig cells were in an activated state in response to FSH. It appears that HA can directly modulate the FSH action on Leydig cells in wall lizards. However, basal levels of Leydig cell steroidogenesis remain unaffected as HA had no effect on testosterone production from non-stimulated Leydig cells. In parallel to our results, the *in vitro* study using purified rat Leydig cells and murine Leydig cell line (MA-10) demonstrates the concentration-dependent biphasic effects of HA on steroidogenesis, though HA modulated both basal and human chorionic gonadotropin (hCG)-induced steroidogenesis (Mondillo *et al.* 2005). Contradictory observations are reported in hamster using testicular parenchyma. HA at high concentration is shown to stimulate the testosterone production, while it has no effect at low concentration

(Mayerhofer *et al.* 1989). From this study, however, it is not evident that HA directly exerted its effect on the Leydig cells and/or indirectly through other cell types present in the testicular parenchyma. The involvement of other cells/endocrine organs in modulating the effect of HA on Leydig cell steroidogenesis further came into picture when *in vivo* (Pap *et al.* 2002) and *in vitro* (Mondillo *et al.* 2006) studies on histidine decarboxylase (*Hdc*) gene knockout mice showed contradictory results. The purified Leydig cells from *Hdc*^{-/-} mice secrete low testosterone (Mondillo *et al.* 2006), while plasma and tissue testosterone levels in *Hdc*^{-/-} mice remain significantly high (Pap *et al.* 2002) when compared with wild-type mice. Despite controversies, based on the results of the present study and the reports in mammals, it is evident that HA does regulate the testicular steroidogenic function. Interestingly, HA at any concentration did not influence the proliferation of Leydig cells in wall lizards, suggesting that HA effect on Leydig cell steroidogenesis might be due to altered steroidogenic enzymes level rather than increase in Leydig cell number. Moreover, no other report is available regarding the effect of HA on Leydig cell proliferation in any of the vertebrate group.

Like Leydig cells, testicular macrophages of wall lizards responded to HA differentially depending upon its concentration. The phagocytosis and superoxide production decreased at high concentrations of HA. At low concentration, the superoxide production increased, while phagocytosis remained unaffected probably due to the fact that machinery involved in phagocytosis might be less sensitive than that in superoxide production to HA. From these results, it is evident that testicular HA is physiologically important in the local control of testicular macrophage immune responses, and thus maintaining testicular immune balance in wall lizards. Although the physiological role for HA in control of testicular immune balance has not been explored so far, it is shown to inhibit chemotaxis, phagocytosis, and superoxide production by rat peritoneal macrophages in a dose-dependent manner (Azuma *et al.* 2001) and, in contrast, stimulates lung macrophage immune responses in humans (Triggiani *et al.* 2001). The possible explanation for differential effects of HA on testicular physiology has been put forward in the present study using selective HA antagonists.

The data herein provide evidence that different HArgic receptors were involved in mediating the concentration-related dual effects of HA on Leydig cell steroidogenesis and testicular macrophage immune responses in wall lizards. The inhibition of steroidogenesis at high concentration of HA was selectively antagonized only by H1 antagonist, pyrilamine, while H2 receptors were seen to be involved in mediating the stimulatory effect of HA at low concentration, as the effect was reversed only by the H2 antagonist, F. Similar observations were made by Mondillo *et al.* (2005) in purified rat Leydig cells and MA-10 Leydig cell line. The occupancy of H2 receptors is shown to activate the adenylate cyclase system, while the H1 receptor leads to the generation of inositol triphosphate (IP₃) (Mondillo

et al. 2005), which, in turn, stimulates calcium release from endoplasmic reticulum. With regard to HA receptors in mediating concentration-related biphasic effects of HA on lizard testicular macrophages, the results were comparable with that of Leydig cells. The H1 receptor antagonist counteracted the effect of HA observed at high concentration, while the effect at low concentration was blocked by the H2 antagonist and not H1. The presence of HA receptors has not been documented on testicular macrophages in any of the vertebrate group. However, at variance to our results, the H2 receptor has been shown to be responsible for the inhibitory effect of HA at high concentration on immune responses of macrophages from tissues other than testis (Azuma *et al.* 2001).

In conclusion, HA differentially regulates the Leydig cell steroidogenesis and testicular macrophage immune responses in wall lizards through selective HA receptors (H1 or H2), and thus contributes directly to the homeostatic control of testicular physiology in a paracrine manner.

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