

Human mast cells express androgen receptors but treatment with testosterone exerts no influence on IgE-independent mast cell degranulation elicited by neuromuscular blocking agents

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Abstract: Women predominate in the anaphylactic reactions to neuromuscular blocking agents (NMBA). The expression of oestrogen receptors has been demonstrated in mast cells and oestrogen treatment can enhance mast cell degranulation, but the influence of androgens remains largely unclear. Our immunocytochemical study showed the expression of androgen receptor (AR) in mast cells isolated from human foreskin as well as in two human mast cell lines, HMC-1 and LAD2. The amount of AR was most abundant in human skin mast cells as determined by real-time polymerase chain reaction analysis. Treatment of the

HMC-1 mast cells with testosterone or 17 β -oestradiol, alone or in combination with different NMBA, did not affect mast cell degranulation as measured by the release of β -hexosaminidase. Our study shows for the first time the expression of AR in human skin mast cells. Further studies using primary human mast cell cultures are needed to understand whether and how sex hormones can influence mast cell activation.

Key words: androgen receptor – mast cell – neuromuscular blocking agent – oestrogen receptor – testosterone

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Background

Anaphylactic reactions occur in 1 in 1000–20 000 general anaesthetics, in which 70% of the adult patients are women (1,2). In both adults and children, the most common elicitors are postsynaptic neuromuscular blocking agents (NMBA) (60%), followed by latex (15–20%) (3,4). The female preponderance was observed in both IgE-mediated and IgE-independent anaphylactic reactions caused by NMBA, with suxamethonium, rocuronium and vecuronium being the most frequently incriminated drugs (1–5).

Many experimental studies show that steroid sex hormones can influence the immune response and allergy development, in which oestrogen is able to enhance humoral immune response and antibody synthesis, whereas androgen seems to exhibit an inhibitory effect (6–8). The expression of oestrogen receptor (ER)- α has been demonstrated *in vivo*

in nasal mucosal mast cells and *in vitro* in the human mast cell line HMC-1, in the rat basophilic leukaemia cell line RBL-2H3, and in primary cultures of bone marrow-derived mast cells from C57BL/6 mice (9,10). The expression of androgen receptor (AR) in mast cells remains ill-defined and not much is known about the effect of testosterone (T) on mast cell degranulation and histamine release.

Questions addressed

The aim of this study was (i) to examine the AR expression in primary skin mast cells (PSMC) and human mast cell lines and (ii) to test if female predominance in anaphylactic reaction to NMBA is associated with the influence of sex hormones on mast cell degranulation.

Experimental design

Isolation of human skin mast cells

Foreskin obtained from circumcision was cut into small pieces and incubated with 0.05% thermolysin type X protease (Sigma-Aldrich, Munich, Germany) in PBS

Abbreviations: AR, androgen receptor; E2, 17 β -oestradiol; ER, oestrogen receptor; FCS, foetal calf serum; IMDM, Iscove's Modified Dulbecco's Medium; NMBA, neuromuscular blocking agent; T, testosterone.

overnight. The dermis was separated from epidermis, digested in an Roswell Park Memorial Institute (RPMI) solution (Invitrogen, Karlsruhe, Germany) containing 10% foetal calf serum (Invitrogen), 0.1% collagenase (Sigma-Aldrich), 0.1% hyaluronidase (Sigma-Aldrich) and 0.5 mg/ml DNase (Sigma-Aldrich) for 3 h at room temperature then agitated. The tissue debris underwent repeated centrifugation, filtration and wash, and mast cells were isolated with anti-human CD117 MicroBeads according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany), attaining a purity at $96.0 \pm 1.3\%$.

We also examined two human leukaemia-derived mast cell lines HMC-1 (11) and LAD2 (12). For positive control in detection of AR expression, the carcinomatous pulmonary epithelial cell line A549 and the human immortalized SZ95 sebocytes were used in PCR study and immunocytochemistry, respectively (13,14), with two human AR-negative prostate cancer cell lines PC3 and DU145 used as negative controls.

Qualitative and quantitative analysis of AR and ER- α expression in human mast cells

Immunocytochemistry on cytocentrifuged mast cells was performed to detect the AR and ER- α proteins. To determine the mRNA expression of AR and ER, a semiquantitative real-time PCR system (TagMan[®] Gene Expression Assay, AR-HS00171172_m1 and ESR1-HS00174860_m1, respectively, Applied Biosystems, Foster City, CA, USA) was performed using 30 ng cDNA as substrate, with 18sRNA as internal control.

Cell treatment with different NMBA

The culture medium was changed to phenol-red free Iscove's modified dulbecco's medium containing charcoal/dextran treated foetal bovine serum (Hyclone Laboratories Inc., Logan, UT, USA) for the last 48 h of culture and throughout the experiments, as described before (10). HMC-1 mast cells (2×10^5 in one sample) were then treated with T or 17 β -oestradiol (E2) (both from Sigma-Aldrich) (solved in ethanol to a final concentration of 10^{-7} M) for 1 h or 72 h, followed by individual stimulation with atracurium besylate (10^{-3} M; Hameln Pharma, Hameln, Germany), pancuronium bromide (10^{-3} M; DeltaSelect GmbH, Pfullingen, Germany), vecuronium bromide (10^{-3} M; N.V. Organon, Oss, The Netherlands), suxamethonium chloride (10^{-2} M; Nycomed Austria GmbH, Linz, Austria) (all solved in 0.9% normal saline) or ionomycin (10^{-3} M; Sigma-Aldrich) (solved in Tyrode's) for 1 h. The compared groups included cells treated with NMBA without prior hormone treatment, or cells treated with sex hormones only. The effect of T or E2 on NMBA-induced mast cell degranulation was examined by measuring the release of β -hexosaminidase into media (15), which was expressed as the percentage of the total amount of β -hexosaminidase that was originally present in the cells.

Statistical analysis

All the experiments were performed in triplicates and repeated thrice. Statistical analysis was performed by two-tailed Student's *t*-test. A *P*-value of <0.05 was considered as statistically significant.

Results

The immunocytochemical study demonstrated the expression of AR (Fig. 1) and ER (data not shown) in the examined mast cells, with the immunostaining localized mainly in the cytoplasm. In real-time PCR, the expression of AR and ER- α was calibrated by the amount of 18sRNA and expressed as delta Ct value (dCt), with lower values representing higher expression levels (16). In PSMC, the ER- α level (dCt = 20.16) was at least fourfold higher than AR level (dCt = 22.57), whereas in HMC-1 the ER- α level (dCt = 22.12) was 16-fold higher than AR level (dCt = 26.57). On the contrary, the LAD2 cells displayed a fourfold higher expression of AR (dCt = 26.24) than ER- α (dCt = 28.06).

In the absence of hormones, only vecuronium at 10^{-3} M (a concentration equivalent to undiluted clinical testing dose) showed a stimulatory effect of 82% on the release of β -hexosaminidase among the examined NMBA, as compared with the control group without any treatment ($P = 0.024$) (Fig. 2a). No stimulation was observed for any NMBA at final concentrations below 10^{-4} M (data not shown), corresponding grossly to a dilution of 1:10 to 1:100 for clinical testing. Atracurium at 10^{-3} M displayed a weak stimulatory effect of 38% ($P = 0.068$), whereas suxamethonium and pancuronium at 10^{-3} M did not show any stimulating effect. Treatment of the cultured HMC-1 mast cells with T or E2 at 10^{-7} M in the absence of NMBA,

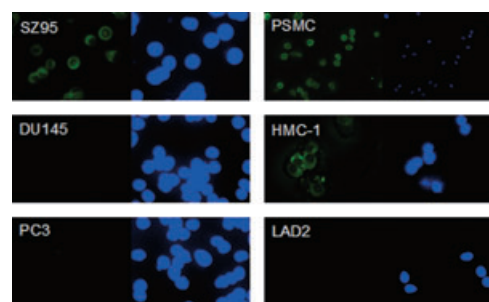


Figure 1. Immunocytochemistry using mouse anti-human androgen receptor monoclonal IgG antibody (Dianova, Hamburg, Germany) at a dilution of 1:25 showed positive immunostaining in SZ95 sebocytes (positive control), primary skin mast cells (PSMC) and HMC-1 mast cells, with scanty expression in LAD2 mast cells and negative findings in PC3 and DU145 cells. The expression of AR was presented with a green-fluorescence (Alexa Fluor[®] 488; Invitrogen, Karlsruhe, Germany), whereas the cell nuclei were stained with a blue-fluorescent dye (DAPI; Invitrogen magnification $\times 400$, Invitrogen, Karlsruhe, Germany).

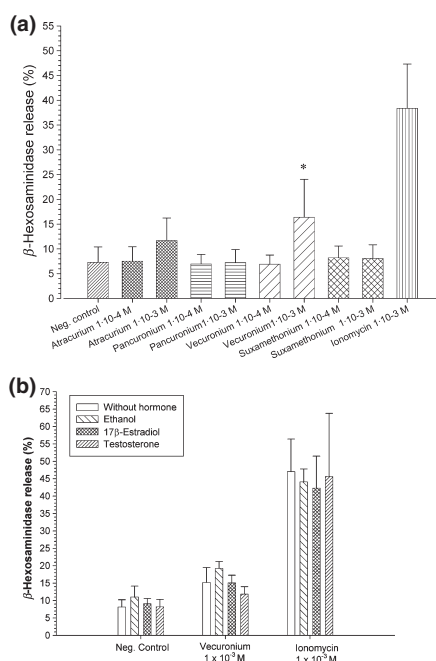


Figure 2. (a) IgE-independent mast cell degranulation after stimulation with neuromuscular blocking agents as measured by the percentage of the released β -hexosaminidase from HMC-1 cells ($n = 3$). As compared with negative control, treatment with vecuronium (10^{-3} M), atracurium (10^{-3} M) or ionomycin (10^{-3} M) for 1 h resulted in an enhancement of 0.8-fold ($P = 0.024$), 0.3-fold ($P = 0.068$) and 4-fold ($P = 0.037$), respectively. No effect was seen with pancuronium or suxamethonium at concentrations of 10^{-3} M or lower. (b) Treatment of the HMC-1 mast cells with testosterone (10^{-7} M) or 17 β -oestradiol (10^{-7} M) alone for 72 h did not alter the degree of mast cell degranulation ($n = 3$). Pretreatment of the cells with testosterone or 17 β -oestradiol for 72 h also failed to affect the degree of mast cell degranulation elicited by vecuronium (10^{-3} M), ionomycin (10^{-3} M) or atracurium (data not shown) ($n = 3$).

for 1 h or 72 h, respectively, did not affect mast cell degranulation. Pretreatment of the mast cells with T or E2 did not affect the β -hexosaminidase release elicited by vecuronium or atracurium (Fig. 2b).

Conclusions

Our results showed for the first time the expression of both AR and ER- α in PSMC. In both PSMC and HMC-1, the ER- α expression was significantly higher than the AR expression. However, in our fluorescence-activated cell sorting analysis, the HMC-1 cells used in the present study, in contrast to PSMC, were lacking of high affinity IgE receptor- α (Fc ϵ RI α) (unpublished data), as described by Butterfield (11). That's why, we could not test the IgE-mediated degranulation of HMC-1 cells induced by NMBA.

Our results were in agreement with the findings derived from an early *in vitro* study using isolated mast cells from

truncal skin, lung and heart, showing that suxamethonium did not induce histamine release from any type of mast cells, whereas atracurium at concentrations higher than 10^{-4} M and vecuronium higher than 10^{-3} M significantly enhanced histamine release from cultured skin mast cells (17). In a clinical skin intradermal testing on healthy anaesthesia-naïve volunteers, the highest non-reactive concentrations to induce a positive wheal formation (≥ 8 mm or doubling of the initial wheal) were 10^{-3} , 10^{-4} , 10^{-4} and 10^{-5} M for suxamethonium, vecuronium, pancuronium and atracurium, respectively (18). In a dermal microdialysis analysis, atracurium was shown to possess a significantly higher efficacy in inducing histamine release in human skin, as compared with suxamethonium, vecuronium or pancuronium (19). Our results differed in that the degree of mast cell degranulation caused by vecuronium at 10^{-3} M was higher, whereas that induced by atracurium was lower, which may be explained by the different types of mast cells tested.

Further studies using cultures of PSMC may help clarify the effect of sex hormones on the IgE-mediated and IgE-independent mast cell degranulation.

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