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Effect of *in vivo* antiestrogen pretreatment on rabbit atrial chronotropic response to histamine

SAMARENDRA N. BAKSI[1] and MAYSIE J. HUGHES[2]

[1]Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology Washington State University Pullman, WA 99164–6520 and [2]Department of Physiology Texas Tech University Health Sciences Center Lubbock, TX 79430

Abstract

The chronotropic response (Δ rate) to histamine (1.4 to 18×10^{-6} *M*) of isolated atria from antiestrogen (tamoxifen)-pretreated immature female rabbit was investigated. Tamoxifen treatment (1.0 and 10.0 mg/kg/day for 14 days) had no significant effect on the Δ rate. The R_{max} and $D_{1/2max}$ were not significantly different in the two tamoxifen-treated groups compared to the oil-treated (1.0 ml/kg/day for 14 days) control group. Cimetidine (2.8×10^{-7} *M*) inhibited the Δ rate to histamine in all groups: control, 27%; tamoxifen (1.0 mg/kg), 38%; and tamoxifen (10.0 mg/kg), 28%. Only the low dose of tamoxifen was found to be estrogenic (uterotropic). We conclude that tamoxifen pretreatment, both at estrogen-agonist and estrogen-antagonist doses, is without effect on atrial chronotropic response to histamine.

Introduction

Histamine, a ubiquitous constituent of normal animal tissues [1, 2], is present in all areas of the mammalian heart in measurable quantities [3] but is highly concentrated in the right atrium [4]. The occurrence of large amounts of histamine in proximity to the specialized center of conduction and automacity [5] and the presence of histamine receptors in atria (6–9) have stimulated a great deal of interest and research regarding the role of histamine in cardiovascular functions [10–18]. The relationship of the presence of histamine in the mammalian heart [2, 3] to the presence of histamine receptors varies among species, as does the cardiac response to histamine [1, 19, 20].

Certain aspects of cardiovascular functions can be altered by estrogens [21, 22]. Estrogens have been shown to modulate histamine receptors at the hypothalamic level [23] and are suspected of altering histamine metabolism [24, 25]. In a previous study [26] we reported that chronic estradiol treatment of immature female rabbit modulates the atrial chronotropic response to histamine, indicating that estrogen can also influence cardiac histamine receptors. Antiestrogens are being increasingly used as therapeutic agents in certain types of human breast cancer [27]. The effects of these drugs on cardiovascular functions are largely unknown. Furthermore, the effects of estrogen and antiestrogen on brain transmitters in rats and rabbits are not always predictable based on the estrogen agonist and antagonist activity [28–30]. The purpose of the present study was to determine if chronic antiestrogen treatment alters cardiac response to histamine in isolated atrial pairs in rabbits.

Methods

Animals

One-month-old (average body weight 1.7 ± 0.2 kg) female New Zealand rabbits were obtained from Bell Rabbit Ranch, Lubbock, TX. The rabbits were fed a commercial diet (Thriftymaster™ Rabbit Pellets, Acco Feeds, Abilene, TX). Tap water was available *ad libitum*. The animal room temperature was 26°C and the light/dark period was controlled with 14 hours of light (light on at 03.00 hr). The animals were acclimatized for one week before the start of the experiment.

Drugs

Tamoxifen citrate (ICI 46,474; p-β-dimethylaminoethoxyphenyl-1,2-diphenyl but-1-ene, lot No. BX 79/NXA/45) used in this study was a gift from ICI Americas. The drug was dissolved in corn oil and injected subcutaneously daily for 14 days. The control group received an equal volume (1.0 ml/kg) of corn oil. The treatment groups and doses were as follows: control (oil); tamoxifen (1.0 and 10.0 mg/kg). Each group contained 6 animals.

Procedures

At the end of the 14-day treatment (52 days after birth) and 24 hours after the final drug or vehicle treatment, each rabbit was decapitated, the chest was opened along the midline, and the whole heart was removed and immersed in Tyrode's solution (Na^+ 146, K^+ 5.6, Mg^{2+} 1.7, Ca^{2+} 1.8, Cl^-

133.6, HCO_3 25.0 and glucose 9.1 in mM/L) which was gassed with 95% O_2 and 5% CO_2. The atrial pair was dissected free of all extraneous tissue and a thread was passed through the tip of each auricular apex. The atria were mounted in a 50 ml tissue bath with one atrium tied to a glass hook and the other to a Statham strain gauge (Model UC-3). The average control tension was 0.7 ± 0.04 g. The tissue was allowed to equilibrate for 60–90 min before the experimental procedure was begun. Signals from transducers were recorded on a Beckman Biomedical Dynograph R411 recorder. Histamine diphosphate (Sigma Chemical Co.) and cimetidine hydrochloride (Tagamet™, Smith Kline and French Laboratories) were dissolved in deionized water and dispensed with a Finnpipette in a volume of 500 µl or less near the base of the bath. The gas mixture, which was bubbled into the tissue bath chamber through a sintered-glass base, served as a source of oxygenation, regulation of pH, and rapid mixing of the drug solutions with the bathing medium. The pH of the bath was 7.4 ± 0.02 and the temperature 36°C. The method has previously been described in detail [31]. Cumulative histamine doses (0.15, 0.25, 0.5, 0.75, 1.0, and 2.0 µg/ml or 1.4, 2.2, 4.5, 6.7, 9.0, and 18.0 × 10^{-6} *M*) were added to the bath at 10-min intervals and the atrial rate was recorded (1-min counts were made before each addition and a 10-min count after the last addition). After the initial histamine dose-response testing, the baths were completely emptied by suction and refilled three times. Retesting did not start until the atrial rate had returned to the control value. After a waiting period of 25 min, the same concentrations of histamine were added using the above method.

Statistics

Statistical calculations were carried out on a Texas Instruments Programmable 59 calculator equipped with applied statistical software. Scratchard plots of the data from the histamine dose-response curves before and after cimetidine were calculated for each concentration of histamine by dividing the change in rate of the chronotropic response (Δ rate: beats/min) by the spontaneous atrial rate. The mathematical basis for these calculations using histamine and isolated atrial pairs has been published [32, 33].

Results

The effect of tamoxifen pretreatment on the chronotropic response to histamine of atria is given in Table 1 and Figures 1 and 2. Scratchard plots were drawn using a statistically determined regression line. The theoretical maximum increase in rate (R_{max}) was found to be 134, 126, and 131 beats/min for the two tamoxifen-treated (1.0 and 10.0 mg/kg) groups and the oil-treated control group (Figure 3), respectively. The treatment caused no significant difference in R_{max}; there was no significant difference in Δ rate after histamine in any group. Cimetidine (2.8 × 10^{-7} *M*), a H_2 receptor antagonist, reduced the maximum rate to 38% in the group treated with the lower dose of tamoxifen compared to 27 and 28% for the oil-treated control group and the group treated with the higher dose of tamoxifen, respectively. The dose of histamine which would cause a half-maximum increase in rate ($D_{1/2max}$) was not significantly different either before or after cimetidine. The slopes and correlation coefficients (γ) for these three groups were calculated and found to be −2.55 (γ = −0.99) for the control group, −1.75 (γ = −0.95) for the 1.0 mg/kg and −2.19 (τ −0.98) for the 10.0 mg/kg tamoxifen-treated group. The mean frequencies of atria for the control, 1.0, and 10.0 mg/kg tamoxifen-treated groups before adding drugs were 133 ± 6, 121 ± 5, and 120 ± 6, respectively, which were not significantly different. Fourteen days' treatment with the lower dose (1.0 mg/kg) of tamoxifen significantly ($p < 0.05$) increased the uterine weight from values for the oil-treated control group: 0.75 ± 0.04 g/kg body weight (mean ± SEM) to 2.14 ± 0.2. This estrogenic effect was absent in the group treated with the higher dose (10.0 mg/kg) of tamoxifen; uterine weight was 0.74 ± 0.05. There was no significant difference

Table 1

The chronotropic response to histamine (1.4 to 18 × 10^{-6} *M*) of isolated atria from immature rabbits pretreated with oil (control) or tamoxifen (Tam) for 14 days. $D_{1/2max}$ is the dose of histamine necessary to obtain the theoretical half of the maximum response. Mean ± SEM of 6 atria.

Treatment	Maximum Δ rate (beats/min)			Histamine dose (× 10^{-6} *M*) for $D_{1/2max}$		
	Before cimetidine (2.8 × 10^{-7} *M*)	After	% Change	Before cimetidine (2.8 × 10^{-7} *M*)	After	%Change
Control (oil)	113 ± 3	83 ± 4**	27	3.5	7.8	55
Tam (1.0 mg/kg)	110 ± 4	68 ± 7**	38	5.2	9.8	48
Tam (10.0 mg/kg)	105 ± 3	76 ± 7*	28	4.1	10.2	60

*$p < 0.01$; **$p < 0.001$. Significantly different from the Δ rate obtained after cimetidine was added to the tissue bath. There was no significant difference in Δ rate due to tamoxifen pretreatment.

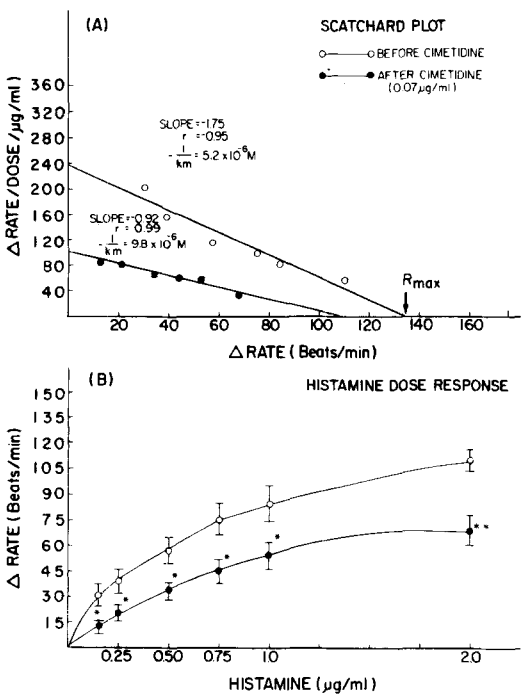

Figure 1
(A) Scatchard analysis of the chronotropic response to histamine of atria from rabbits pretreated with low dose of tamoxifen (1.0 mg/kg/day) for 14 days. Data from histamine dose-response curve. R_{max} is the maximum calculated response and can be read from horizontal axis. The slope yields $-1/km$.
(B) Rabbit atrial chronotropic response to histamine (1.4 to 18×10^{-6} *M*) from rabbits pretreated with low dose of tamoxifen. Low curve depicts chronotropic response after addition of cimetidine (2.8×10^{-7} *M*) in the tissue bath. Mean ± SEM (vertical bar) of six atrial pairs. Significantly different: *$p < 0.05$; **$p < 0.01$.

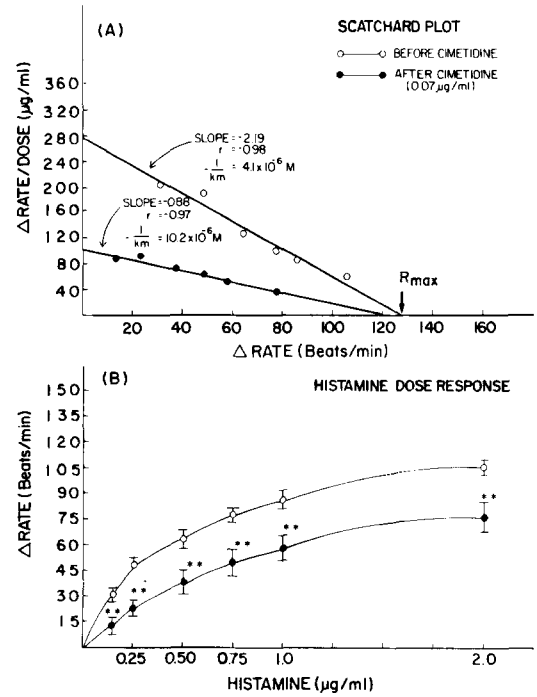

Figure 2
(A) Scatchard analysis of the chronotropic response to histamine of atria from rabbits pretreated with high dose of tamoxifen (10.0 mg/kg/day) for 14 days. See Fig. 1 for other notations.
(B) Rabbit atrial chronotropic response to histamine from rabbits pretreated with high dose of tamoxifen. See Fig. 1 for other notations.

in body weight gain due to the treatments (data not shown).

Discussion

The present study demonstrates that pretreatment with an antiestrogen (tamoxifen) does not significantly alter the number of histamine receptors in the atria, as evident from the chronotropic response study . The maximum Δ rate after histamine was not significantly altered by pretreatment with tamoxifen or a tenfold dose of tamoxifen. The inhibition of R_{max} after cimetidine, a H_2 receptor antagonist, was less pronounced (5% inhibition) in atria treated with the higher dose of tamoxifen than in the oil-treated control group or in atria treated with the lower dose of tamoxifen (20% inhibition). The change in Δ rate after histamine is assumed to be due to the change in the number of histamine receptors in the atria. Rabbit atria contain both H_1 and H_2 chronotropic histamine receptors [14, 34], The respective $D_{1/2max}$, calculated from the slope of the regression line, was also not significantly different due to the treatment. It is assumed that there was no change in the number and affinity of the receptor population. If the affinities of the H_1 and H_2 receptors are very different, and if the receptor type that has the least affinity is reduced or functionally deleted, then the apparent affinity would appear to increase. The probable reduction in the number of receptor sites and lower affinity after the addition of cimetidine, a H_2 type receptor antagonist, suggest that H_1 type receptors should not have been affected. The R_{max} of atria in the control rabbits after cimetidine blockade was 116 beats/min compared to 131 beats/min before cimetidine, a 12% reduction. This suggests non-

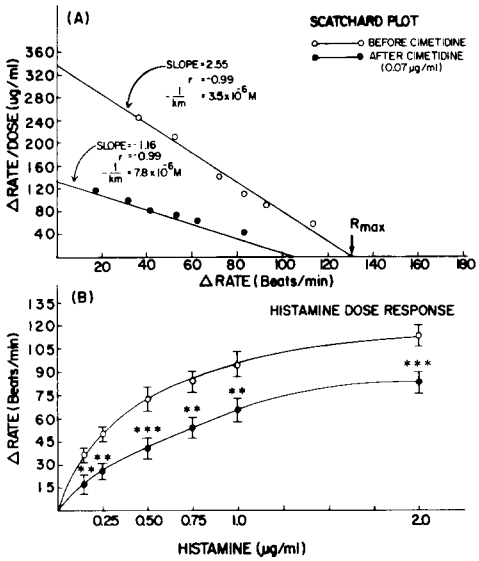

Figure 3
(A) Scatchard analysis of the chronotropic response to histamine of atria from rabbits pretreated with oil (1.0 ml/kg/day) for 14 days. See Fig. 1 for other notations.
(B) Rabbit atrial chronotropic response to histamine from oil-treated (control) rabbits. See Fig. 1 for other notations. ***$p < 0.001$.

competitive inhibition, which could be explained on the basis of the presence of H_1 and H_2 receptors. It is not known whether histamine has a preferential affinity for H_1 or H_2 receptor sites; however, one might speculate that some difference may exist. If there are rather nonspecific histamine receptors, their affinity would probably be between those of the specific H_1 and H_2 type. A recent study indicates that tamoxifen specifically blocks the histamine-induced (H_1 type receptor) contraction of canine tracheal smooth muscle *in vitro* [35], showing that tamoxifen is a histamine antagonist of H_1 type receptors. It is less evident in our study whether a 14-day treatment with tamoxifen had any effect at the H_1 receptor sites, except that there was a less pronounced inhibition of R_{max} after cimetidine in atria treated with the highest dose of tamoxifen. It is possible that tamoxifen treatment at this dose affected the affinity of the H_1 receptors in the atria. The lower dose of tamoxifen (1.0 mg/kg), which was uterotropic and hence acted as an estrogen agonist, did not have the same effect as estradiol on atrial histamine response [26]. Estrogen treatment has been shown to reduce the histamine content of rat uterus [36] and to increase the releasable fraction of histamine from guinea pig atria [37]. It is not known if tamoxifen, a non-steroidal antiestrogen, acts like estrogen in the rabbit atria when administered in low doses. Antiestrogens have some intrinsic estrogen-agonist activity on estrogen-target organs in some species [38–40]. The estrogenic activity of low-dose tamoxifen on rabbit uterus and the apparent absence of an effect on histamine receptors in the atria suggest that antiestrogen has different functions in the uterus and atria of rabbit. The exact molecular mechanism of antiestrogen action has not been worked out [39]. Although rat atria are known to possess estrogen receptors [41], it is not known whether antiestrogen at an agonist dose activates these receptors.

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References
[1] M. A. BEVEN, *Histamine: its role in physiological and pathological processes*, pp. 1–113. S. KARGER, Basel, New York 1978.
[2] W. LORENZ, E. MATEJKO, A. SCHMAL, W. SEIDEL, H-J REIMAN, R. UHLIG and G. MANN, *A phylogentic study of the occurrence and distribution of histamine in the gastrointestinal tract and other tissues of man and various animals*, Comp. Gen. Pharmacol. *4*, 229–250 (1973).
[3] P. F. MANNAIONI, *Physiology and pharmacology of cardiac histamine*, Arch. Int. Pharmacodyn. Ther. *196 [Suppl.]*, 64–78 (1972).
[4] A. GIOTTI, A. GUIDOTTI, P. F. MANNAIONI and L. ZILLETTI, *The influence of adrenotropic drugs and noradrenaline on the histamine release in cardiac anaphylaxis in vitro*, J. Physiol. *184*, 924–941 (1966).
[5] J. P. TRZECIAKOWSKI and R. LEVI, *Cardiac histamine: a mediator in search of a function*, Trends Pharmacol. Sci. *2*, 14–17 (1981).
[6] M. J. HUGHES and I. A. CORET, *On specificity of histamine receptors in the heart*, Am. J. Physiol. *223*, 1257–1262 (1972).
[7] D. REINHARDT, J. WAGNER and H. J. SCHUMANN, *Differentiation of H_1- and H_2-receptors mediating positive chronotropic and inotropic responses to histamine on atrial preparation of the guinea pig*, Agents and Actions *4*, 217–221 (1974).
[8] S. C. VERMA and J. H. MCNEILL, *Cardiac histamine receptors: differences between left and right atrium and right ventricle*, J. Pharmacol. Exp. Ther. *200*, 352–362 (1977).
[9] R. LEVI, N. CAPURRO and C. H. LEE, *Pharmacological characterization of cardiac histamine receptors: sensitivity to H_1- and H_2-receptor agonists and antagonists*, Europ. J. Pharmacol. *30*, 328–335 (1975).

[10] W. FLACKE, D. ATANACKOVIC, R. A. GILLIS and M. H. ALPER, *The actions of histamine on the mammalian heart*, J. Pharmacol. Exp. Ther. *155*, 271–278 (1967).

[11] B. M. ALTURA and S. HALEVY, Cardiovascular actions of histamine, In: *Handbook of Experimental Pharmacology*, vol. 18/2, pp. 1–39 (Eds G. V. R. BORN, O. EICHLER, A. FARAH, H. HERKEN and A. D. WELCH) Springer-Verlag, Heidelberg, New York 1977.

[12] M. J. HUGHES and I. A. CORET, *A quantitative study of histamine H_2-receptor blockade by burimamide in isolated atria*, Proc. Soc. Exp. Biol. Med. *148*, 127–133 (1975).

[13] J. H. MCNEILL, S. C. VERMA and T. E. TENNER, JR, Cardiac histamine receptors. In: *Advances in Myocardiology*, vol. 1, pp. 209–216 (Eds M. TAJUDDIN, P. K. DAS, M. TARIQ and N. S. DHALLA) University Park Press, Baltimore 1980.

[14] M. J. HUGHES, *A question of the specificity of rabbit atrial chronotropic histamine receptors and agents which affect their activity*, J. Clin. Pharmacol. *20*, 10–19 (1980).

[15] R. LEVI, G. ALLAN and J. H. ZAVECZ, *Cardiac histamine receptors*, Fed. Proc. *35*, 1942–1947 (1976).

[16] D. A. A. OWEN, *Histamine receptors in the cardiovascular system*, Gen. Pharmacol. *8*, 141–156 (1977).

[17] T. E. TENNER, JR, *Comparison of H_2 receptor agonists in rabbit atria and aorta*, Pharmacology *22*, 227–234 (1981).

[18] K. SAKAI, *Role of histamine H_1- and H_2-receptors in the cadiovascular system of the rabbit*, J. Cardiovas. Pharmacol. *2*, 607–617 (1980).

[19] O. B. REITE, *Comparative physiology of histamine*, Physiol. Rev. *52*, 778–819 (1972).

[20] R. LEVI, D. A. A. OWEN and J. P. TRZECIAKOWSKI, *Actions of histamine on the heart and vasculature*. In: Pharmacology of Histamine receptors, pp. 236–297 (Ed. C. R. GANELLIN and M. E. PARSONS) Wright PSG, Bristol 1982.

[21] R. J. BEARD, *Estrogens and the cardiovascular system*. In: The Menopause, A Guide to Current Research and Practice, pp. 82–94 (Ed. R. J. BEARD) University Park Press, Baltimore 1976.

[22] B. V. STADEL, *Oral contraceptives and cardiovascular disease*, New Eng. J. Med. *305*, 612–618 and 672–677 (1981).

[23] P. PORTALEONE, E. GENAZZANI, G. PAGNINI, A. CRISPINO and F. DICARLO, *Interaction of estradiol and 2-hydroxyestradiol with histamine receptors at hypothalamic level*, Brain Res. *187*, 216–220: (1980).

[24] J. P. GREEN, D. H. FRAM and K. KASE. *Methylhistamine and histamine in the urine of women during elaboration of estrogen*, Nature *204*, 1165–1168 (1964).

[25] F. JONASSEN, G. GRANERUS and H. WITTERQVIST, *Histamine metabolism and female sex hormones in women*, Acta. Obstet. Gynecol. Scand. *55*, 387–394 (1976).

[26] S. N. BAKSI and M. J. HUGHES, *Modulation by estradiol of rabbit atrial chronotropic response to histamine*, Basic Res. Cardiol. *78*, 505–509 (1983).

[27] S. S. LEGHA, H. L. DAVIS and F. M. MUGGIA, *Hormonal therapy of breast cancer: new approaches and concepts*, Ann. Int. Med. *88*, 69–77 (1978).

[28] S. N. BAKSI, T. E. REDINGTON and M. J. HUGHES, *Antiestrogen-induced alterations of hypothalamic dopamine and norepinephrine levels in the female rat*, Neuropharmacology *20*, 1163–1167 (1981).

[29] S. N. BAKSI and M. J. HUGHES, *Alteration of dopamine metabolism in different brain regions of the rabbit by estradiol and tamoxifen*, Neuroscience *14*, 1053–1059 (1985).

[30] V. N. LUINE and B. S. MCEWEN, *Effects of an estrogen antagonist on enzyme activities and ^{3}H-estradiol nuclear binding in uterus, pituitary and brain*, Endocrinology *100*, 903–910 (1977).

[31] M. J. HUGHES, *Direct and indirect actions of impromidine (a new H_2 receptor agonist) on atrial tissue*, Life. Sci. *29*, 817–824 (1981).

[32] I. A. CORET and M. J. HUGHES, *A quantitative description of chronotropic effects of histamine on rabbit heart*, Arch. Int. Pharmacodyn. Ther. *208*, 117–127 (1974).

[33] M. J. HUGHES and I. A. CORET, *A characteristic of the rate response which is common to several compounds that stimulate the heart*, J. Mol. Cell Cardiol. *7*, 613–624 (1975).

[34] J. H. MCNEILL and S. C. VERMA, *Histamine receptors in rabbit heart*, Proc. Western Pharmacol. Soc. *21*, 99–101 (1978).

[35] E. A. KROEGER and L. J. BRANDES, *Evidence that tamoxifen is a histamine antagonist*, Biochem. Biophy Res. Commun. *131(2)*, 750–755 (1985).

[36] T. C. MCKERCHER, L. S. VANORDEN, R. K. BHATNAGAR and J. P. BURKE, *Estrogen induced biogenic amine reduction in rat uterus*, J. Pharmacol. Exp. Ther. *185*, 514–522 (1973).

[37] M. J. CONRAD and G. A. FEIGEN, *Sex hormones and kinetics of anaphylactic histamine release*, Physiol. Chem. Physics. *6*, 11–16 (1974).

[38] S. HELGASON, N. WILKING, K. CARLSTROM, M-G. DAMBER and A. VON SCHOULTZ, *A comparative study of the estrogenic effects of tamoxifen and 17-β estradiol in postmenopausal women*, J. Clin. Endocrinol. Metab. *54*, 404–408 (1982).

[39] V. C. JORDAN, *Biochemical pharmacology of antiestrogen action*, Pharmacol. Rev. *36*, 245–276 (1984).

[40] B. S. KATZENELLENBOGEN, H. S. BHAKOO, E. R. FERGUSON, N. C. LAW, T. TATEE, T. S. TSAI and J. A. KATZENELLENBOGEN, *Estrogen and antiestrogen actions on reproductive tissues and tumors*, Recent Prog. Hormone Res. *35*, 259–300 (1979).

[41] W. E. STUMPF, M. SAR and G. AUMULLER, *The heart: a target organ for estradiol*, Science *196*, 319–321 (1977).