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European Journal of Pharmacology 438 (2002) 45–52

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Intracellular cAMP increases during the positive inotropism induced by androgens in isolated left atrium of rat

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Received 31 May 2001; received in revised form 18 January 2002; accepted 22 January 2002

Abstract

Molecular interactions of androgens with the plasma membrane may produce rapid cardiovascular effects that cannot be explained by the classic genomic mechanisms. In this sense, 5α- and 5β-dihydrotestosterone-induced an acute positive inotropic effect in isolated left atrium of rat, an effect which may be due to cAMP-dependent mechanisms. To prove this, intracellular levels of cAMP, after exposure to androgens in the organ bath, and binding to β_1-adrenoceptors were evaluated. After a 4-min exposure, 5α- and 5β-dihydrotestosterone increased cAMP levels from 3.83 ± 0.61 to 6.15 ± 1.1 and 11.18 ± 2.4 pmol cAMP/mg of protein, respectively. These increases were inhibited by atenolol and not modified by treatment of the rats with reserpine. The androgen-induced cAMP increase seems to be produced via an extracellular interaction, because positive inotropism and raised levels of cAMP were produced by 5α-dihydrotestosterone conjugated with bovine serum albumin (BSA). In addition, it is independent of β_1-adrenoceptor activation, because neither androgen displaced [^{3}H]dihydroalprenolol binding. Therefore, the androgens induced a positive inotropic effect via a postsynaptic effect that increases intracellular levels of cAMP. This effect is modulated by transcriptional mechanisms or by a protein with a short half-life.

Keywords: Androgen; Atrium, rat; cAMP

1. Introduction

Molecular interactions of androgens with the plasma membrane may produce rapid cardiovascular effects that cannot be explained by the classic genomic mechanisms (Falkenstein et al., 2000). In this sense, androgens may produce beneficial hemodynamic effects on the clinical parameters of myocardial ischemia by modulating coronary tone and by relaxing of the aorta (Yue et al., 1995; Costarella et al., 1996), which reduces afterload. Furthermore, 5α- and 5β-dihydrotestosterone induce acute positive inotropism in the isolated left atrium of the rat (García Valencia et al., 1989). This effect may be synergic with the vascular effect, improving the performance of the heart.

Pharmacological evidence shows that cAMP-dependent mechanisms may be related to androgen-induced positive inotropism in the left atrium of the rat. The effect is produced at a postsynaptic level and is antagonized by the β-adrenoceptor antagonists, propranolol and atenolol, and is pertussis toxin sensitive (Rubín et al., 1999). In addition, the positive inotropism seems to involve transcription and protein synthesis, since it is inhibited by actinomycin D and cycloheximide (Rubín et al., 1999).

Cyclic AMP is involved in the rapid activation of transcription, suggesting that cAMP may induce gene expression by modulating the activity of existing nuclear factors (Lewis et al., 1987). Related to this, a rapid and transient induction of c-*fos* was reported to occur in the submandibular gland and heart of the mouse (Barka et al., 1986) after β-adrenoceptor stimulation. The expression of the rapid response gene c-*jun*, besides being β-adrenoceptor-stimulated (Iwaki et al., 1990; Nakamura et al., 1990; Stewart, 1995; Tamir et al., 1996), is regulated by polyamines (Wang et al., 1993; Patel and Wang, 1997; Bjersing et al., 1997). Polyamine synthesis is involved in the rapid positive inotropism elicited by androgens in the left atrium of the rat (Bordallo et al., 2001).

A rapid interaction between androgens and the plasma membrane, involving an increase in cAMP, and stimulation of the modulatory mechanisms of transcription, could play

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01300-6

a functional role in the enhancement of the contractility elicited by androgens in the rat left atrium. Thus, to study this possibility, the intracellular levels of cAMP during exposure to androgens were measured and it was determined whether androgens bind to β_1-adrenoceptors. In addition, Northern blotting analysis was performed to ascertain the level of expression of the genes c-*fos* and c-*jun* during the positive inotropism elicited by acute exposure to androgens and isoproterenol in the left atrium of the rat.

2. Methods

2.1. Preparation of tissue and incubation media

Three-month-old male Wistar rats, 250–300 g in weight (University of Oviedo, Spain, number 3304-13A), were used. These were killed by decapitation under anesthesia with ethyl ether in an inhalation chamber. Afterwards, the left atrium was extracted and placed in an organ bath in 10 ml of Tyrode's solution (having the following mM composition: NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9 and glucose, 5.5) at 37 °C and bubbled continuously with a 95% O_2 and 5% CO_2 mixture.

To avoid the possibility of presynaptic modulatory effects on adrenergic transmission, a group of animals was treated with reserpine 5 $mg \cdot kg^{-1}$ 24 h before being killed (Martínez et al., 1995).

2.2. Experimental procedure

The tissues were allowed to equilibrate for 1 h under a basal tension of 1*g* before experimentation. After this period, electrical stimulation, with a Grass S11 stimulator at 0.5 Hz with a duration pulse of 5 milliseconds (ms) and supramaximal voltage (30–50% above the threshold voltage) was started, allowing a period of 30 min before the administration of any drug to the organ bath. To study the effect of atenolol and isobutyl-methylxanthine (IBMX) on 5α-dihydrotestosterone-elicited modifications of intracellular levels of cAMP, the drugs were added, 5 and 30 min before exposure to the androgen, respectively. Contractions were recorded on a Letica Uni-graph 50 polygraph through isometric transducers TRI 110.

2.3. Cyclic AMP analysis

To determine the basal levels of cAMP, after the incubation period, the atria were electrically stimulated. Since the data are unpaired, one of the five atria placed in the organ baths was considered as control, and the others were exposed to 5α-, 5β-dihydrotestosterone, isoproterenol, atenolol, etc. Afterwards, the preparations were immediately removed from the organ bath and placed in liquid nitrogen. Then, these preparations were preserved at −80 °C until use. To study the effect of androgens on cAMP levels, single concentrations of 5α-dihydrotestosterone (10 and 100 μM), 5β-dihydrotestosterone (100 μM), 5α-dihydrotestosterone conjugated with bovine serum albumin (100 μM), or isoproterenol (0.3 μM) were added to the organ bath. In order to estimate the time course of cAMP modifications, the preparations were removed from the organ bath at different times, from 0.5 to 6 min, and placed in liquid nitrogen.

To determine cAMP levels, the atria were homogenized with a Polytron in buffer containing 4 mM EDTA (to prevent enzymatic degradation of cAMP), followed by heating for several minutes in a boiling water bath, to coagulate the protein. The extracts were centrifuged at 12,000 rpm for 15 min using a Suprafuge 22. The cAMP in the supernatant was assayed following the indications of the manufacturer (Amersham).

2.4. Binding assay

The method was essentially performed as described by Hartmann et al. (1995). Hearts were cleaned of fat and connective tissue, weighed and immediately homogenized in ice-cold buffer (mM: sucrose 0.25, Tris 5, $MgCl_2$ 1; pH 7.4) by using a Polytron. The homogenates were diluted with an equal volume of KCl (1 M), stirred 10 min and filtered through gauze. The homogenate was centrifuged at $700 \times g$ (10 min) and the supernatant was spun at $10{,}000 \times g$ (15 min) and then at $40{,}000 \times g$ (30 min). The membrane pellet was dissolved in buffer (mM: Tris 50, $MgCl_2$ 10; pH 7.4).

The binding assays were carried out in 500 μl, containing 200 μg of membrane protein and a buffer (mM: Tris 50, $MgCl_2$ 10; pH 8), and incubated at 30 °C for 20 min. The bound ligand was separated by rapid vacuum filtration through Whatman GF/B filters. The radioactivity was determined by liquid scintillation.

Cardiac β-adrenoceptor densities (B_{max}) were estimated in saturation experiments using [^{3}H]dihydroalprenolol and specific binding was defined as the portion displaceable by propranolol (1 μM). The androgen binding displacement experiments were performed using increasing concentrations of 5α- or 5β-dihydrotestosterone (0.1–100 μM) in the presence of [^{3}H]dihydroalprenolol (1 nM). All experiments were conducted in triplicate and repeated at least four independent times.

Protein concentrations were determined by the Bio-Rad protein assay (Richmond, CA) and an appropriate quantity of buffer was added to reach a protein concentration of 2 mg/ml.

2.5. Northern blotting analysis

For the Northern blotting analysis, the atrium was placed in the organ bath following the same procedure as

described above. The preparations were exposed for 6 min to 100 μM of 5α-, 5β-dihydrotestosterone or 0.3 μM of isoproterenol and then removed from the organ bath and placed in liquid nitrogen. The tissues were preserved at − 80 °C, until use.

Total RNA was isolated from the tissue by the guanidine isothiocyanate method as previously described (Chomczynsky and Sacchi, 1987). A total of 20 μg of total RNA were fractionated on a 1.2% agarose gel containing 2.2 M formaldehyde, blotted onto nylon membranes Hybond N (Amersham), and fixed with ultraviolet light in an ultraviolet cross-linker. The blots were then prehybridized for 6 h at 42 °C in a solution containing 50% formamide, 5x saline sodium phosphate EDTA (SSPE) (1 × SSPE: 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA pH 7.4), 10 × Denhardt's solution (1 × Denhardt's: 0.1% of each bovine serum albumin (BSA), polyvinyl pyrrolidone and Ficoll), 2% sodium dodecyl sulphate (SDS) and 100 μg/ml denatured salmon sperm DNA. Membranes were hybridized in the same solution with a cDNA encoding rat c-*fos* and c-*jun* labelled with α^{32}PdCTP for 48 h at 42 °C. The blots were washed twice at room temperature in 2 × SSC (1 × SSC: 150 mM NaCl, 15 mM trisodium citrate; pH 7), 0.1% SDS for 5 min each, followed by washing at 50 °C in 0.1 × SSC, 0.1% SDS for 20 min each. Blots were exposed to Kodak X-Omat films at − 70 °C with inten-sifying screens. Following exposure to X-ray film, Northern blots were washed in stripping buffer (0.5% SDS, 5 mM EDTA; pH 8) for 3 min at 95 °C and hybridized again with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA as an internal control to evaluate RNA loading.

2.6. Drugs

The following drugs were used: 5α-dihydrotestosterone (5α-androstan-17-β-ol-3-one), 5β-dihydrotestosterone (etiocholan-17β-ol-3-one), isoproterenol (1-[3′,4′ -Dihydroxyphenyl]-2-isopropyl-aminoethanol, hydrochloride), atenolol (4-[2′ -Hydroxy-3-(isoprylamine)-propoxy] phenylacetamide), IBMX (3-Isobutyl-l-methylxanthine) and reserpine (methyl reserpate 3,4,5-trimethoxy-benzoic acid ester) from Sigma. Cyclic AMP [^{3}H] radioassay kit and [^{3}H]dihydroalprenolol were from Amersham. 5α- and 5β-dihydrotestosterone were dissolved in ethanol (the final concentration of the solvent was 0.1%); 5α-dihydrotestosterone conjugated with bovine serum albumin (BSA) was dissolved in DMSO. Additional experiments were carried out to evaluate the effect of ethanol and DMSO. At the concentrations used, ethanol and DMSO did not facilitate the contractility of the left atria. Isoproterenol was dissolved in double distilled water.

2.7. Calculation and statistical analysis

The data obtained are expressed as the means ± S.E.M. for a number of data (n) of at least 4 in each case. Statistical significance was calculated by means of the Student's t Test for unpaired values, considering $P<0.05$ as significant. A linear regression analysis of Scatchard plot data was made by using the computer radioligand program RADLIG (Biosoft). The K_D and B_{max} (maximal binding capacity related to the number of receptors) were also determined.

3. Results

3.1. Effect of 0.3 μM of isoproterenol on intracellular levels of cAMP in the left atrium of rats

To validate the measurement of intracellular cAMP under our experimental conditions, we performed experiments with isoproterenol, an agonist of β-adrenoceptors, which is known to induce positive inotropism via an increase in the intracellular levels of cAMP. The time course of changes in intracellular cAMP was determined in the atria exposed to 0.3 μM isoproterenol by adding the drug to the organ bath. The cardiotonic effect was recorded and, at different times from 30 s to 3 min, the atria were removed from the organ bath and immediately placed in liquid nitrogen. The tissues were preserved at − 80 °C until used to measure intracellular cAMP.

Isoproterenol 0.3 μM increased the intracellular levels of cAMP after 30 s of exposure from 3.83 ± 0.61 pmol cAMP mg of protein (basal value) to 20.88 ± 3.62 (Fig. 1).

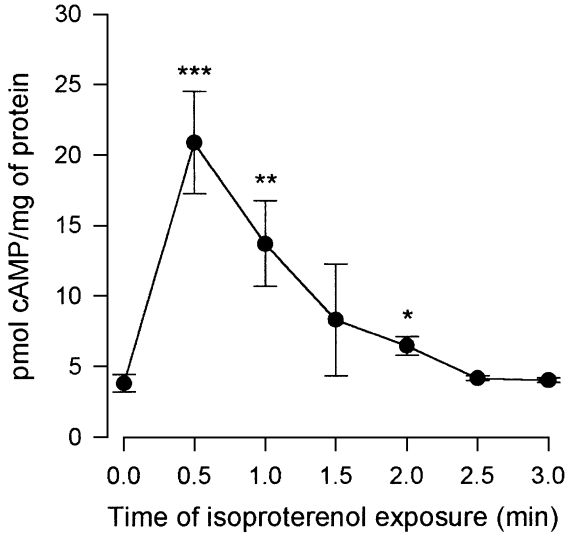

Fig. 1. Intracellular levels of cAMP at different times (from 0.5 to 3 min) after exposure to 0.3 μM isoproterenol of the electrically stimulated (0.5 Hz, 5 ms, voltage 30–50% above threshold voltage) left atrium of rats. cAMP levels are expressed as pmol/mg of protein. Each point represents the mean ± S.E.M. for at least four data. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ by means of Student's t-test with respect to basal levels.

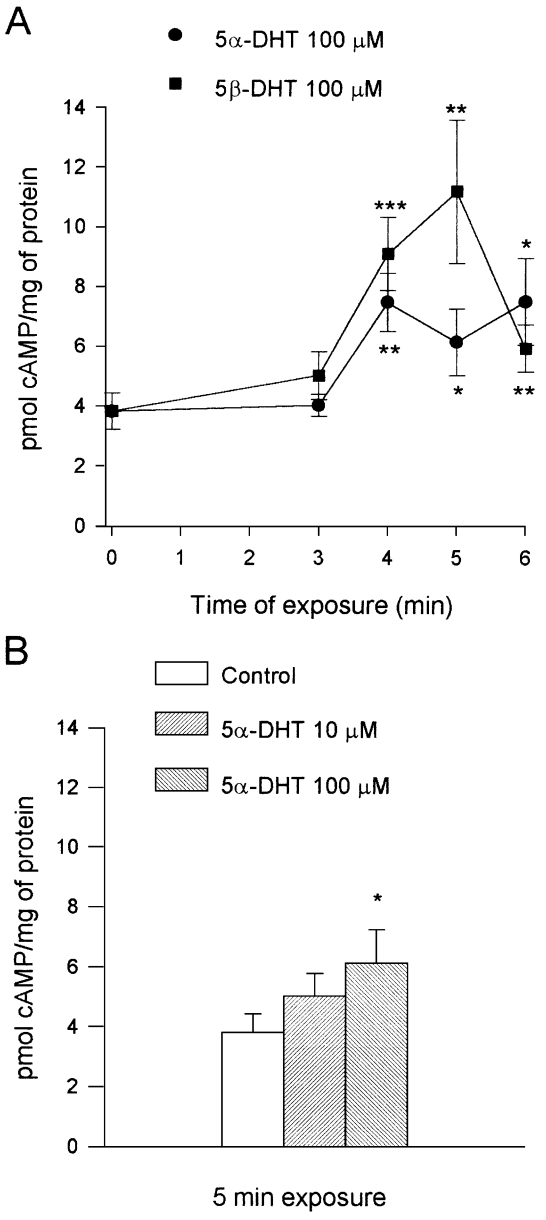

Fig. 2. (A) Effect of different times of incubation (from 3 to 6 min) with 5α- and 5β-dihydrotestosterone (DHT, 100 μM) and (B) of 5 min of exposure to 5α-dihydrotestosterone 10 and 100 μM on intracellular cAMP levels in the electrically stimulated (0.5 Hz, 5 ms, 30–50% above threshold voltage) left atrium of rats. cAMP levels are expressed as pmol/mg of protein. Each point represents the mean ± S.E.M. for at least four data. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ by means of Student's *t*-test with respect to basal levels.

3.2. Effect of acute exposure to 5α- and 5β-dihydrotestosterone on intracellular levels of cAMP in the left atrium of rats

To evaluate the time course of the intracellular modifications of cAMP levels after 5α- or 5β-dihydrotestosterone-induced positive inotropism, these drugs were added at single concentrations (100 μM) to the organ bath, and at 1-min intervals from 1 to 6 min of exposure the atria were removed and placed in liquid nitrogen. Additional experiments were performed with atria without androgen exposure in order to determine the basal levels of intracellular cAMP.

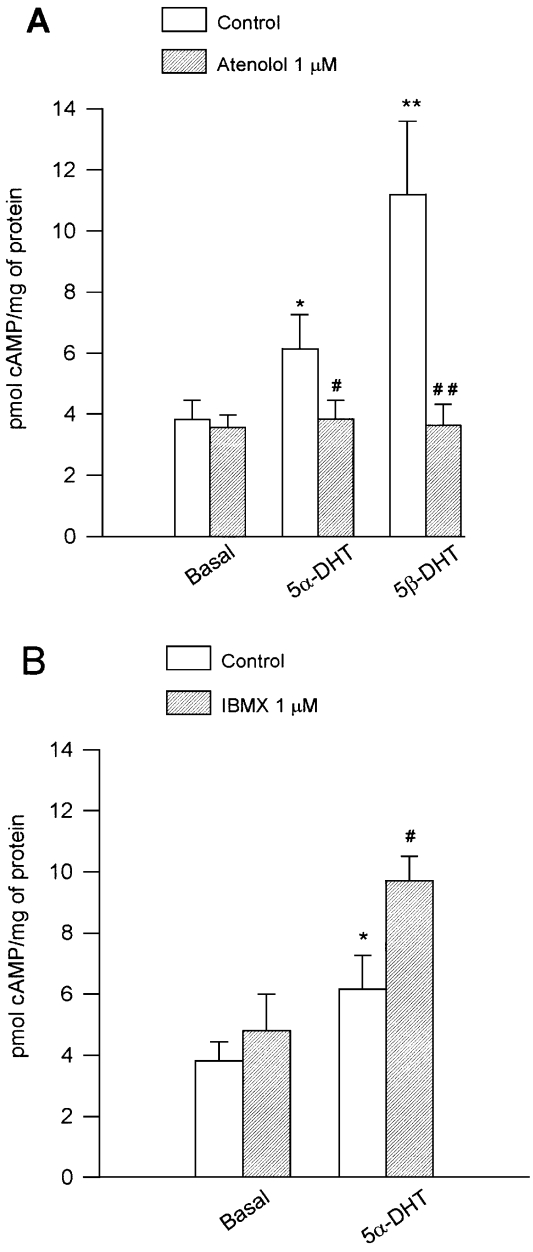

Fig. 3. (A) Effect of 1 μM atenolol and (B) IBMX 10 μM on the increase in intracellular cAMP levels after a 5-min incubation in the organ bath with 5α- and 5β-dihydrotestosterone (DHT, 100 μM) in the left atrium of rats after electrical stimulation (0.5 Hz, 5 ms, 30–50% above threshold voltage). cAMP levels are expressed as pmol/mg of protein. Each point represents the mean ± S.E.M. for at least five data. * $P<0.05$ and * $P<0.01$ by means of Student's *t*-test with respect to basal levels. # $P<0.05$ and ## $P<0.01$ by comparison of 5α- and/or 5β-dihydrotestosterone (DHT, 100 μM)-induced increases in the absence and in the presence of atenolol and IBMX.

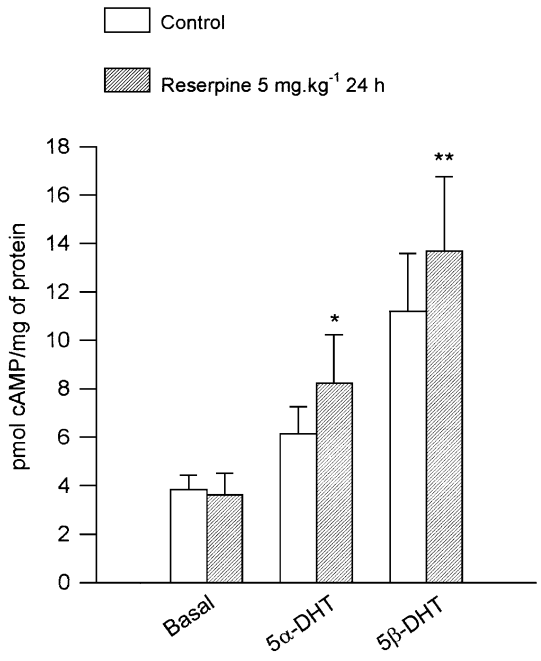

Fig. 4. Effect of reserpine treatment of rats (5 mg·kg^{-1}, 24 h before death) on basal levels of intracellular cAMP and after a 5-min exposure to 5α- and 5β-dihydrotestosterone (DHT, 100 μM) in electrically stimulated (0.5 Hz, 5 ms, 30–50% above threshold voltage) left atrium. cAMP levels are expressed as pmol/mg of protein. Each point represents the mean ± S.E.M. for at least five data. * $P<0.05$ and * * $P<0.01$ by means of Student's *t*-test with respect to basal levels obtained for reserpine-treated rats.

After 4 min of exposure, the androgens induced a significant increase in cAMP levels, an effect which was sustained without significant change for 5 and 6 min for 5α-dihydrotestosterone and which was significantly higher than basal values for 5β-dihydrotestosterone (Fig. 2A).

5α-Dihydrotestosterone (10 μM) increased intracellular levels of cAMP, although less than 100 μM did (Fig. 2B).

3.3. Effect of 1 μM atenolol on intracellular levels of cAMP in the left atrium of rats after acute exposure to 100 μM 5α- and 5β-dihydrotestosterone

To study the effect of a β-blocker, atenolol, the variations in intracellular levels of cAMP after 5 min of androgen exposure were determined. Atenolol (1 μM), as described (Rubín et al., 1999), significantly inhibited the contraction induced by both 5α- and 5β-dihydrotestosterone and the increase in intracellular cAMP levels (Fig. 3A).

3.4. Effect of 10 μM IBMX on intracellular levels of cAMP in the left atrium of rats after acute exposure to 100 μM 5α- and 5β-dihydrotestosterone

To study the effect of an inhibitor of phosphodiesterase on the intracellular levels of cAMP, the preparations were incubated with IBMX (10 μM), and after 5 min of exposure to 5α-dihydrotestosterone, the intracellular level of cAMP was determined. IBMX did not modify the basal levels of intracellular cAMP, but significantly increased the intracellular levels of cAMP after 5α-dihydrotestosterone exposure (Fig. 3B).

3.5. Effect of the reserpine treatment of rats on cAMP levels after in vitro exposure to 100 μM 5α- and 5β-dihydrotestosterone in the left atrium

Treatment of the rats with reserpine according to a protocol that pharmacologically excludes presynaptic modulatory mechanisms at adrenergic terminals (Martínez et al., 1995; Rubín et al., 1999) did not prevent the androgen-induced contractions nor did it modify the increase in cAMP levels after the addition of 100 μM androgens to the organ bath (Fig. 4).

3.6. Effect of 100 μM 5α-dihydrotestosterone conjugated with bovine serum albumin on basal inotropism and intracellular levels of cAMP in the left atrium of rats

To study the possibility that 5α-dihydrotestosterone had extracellular effects, an equimolar concentration (100 μM) of the androgen conjugated with bovine serum albumin (BSA) was added to the organ bath to assay whether this modified basal inotropism and intracellular cAMP levels in the rat left atrium. 5α-Dihydrotestosterone–BSA significantly increased the positive inotropism, although the per-

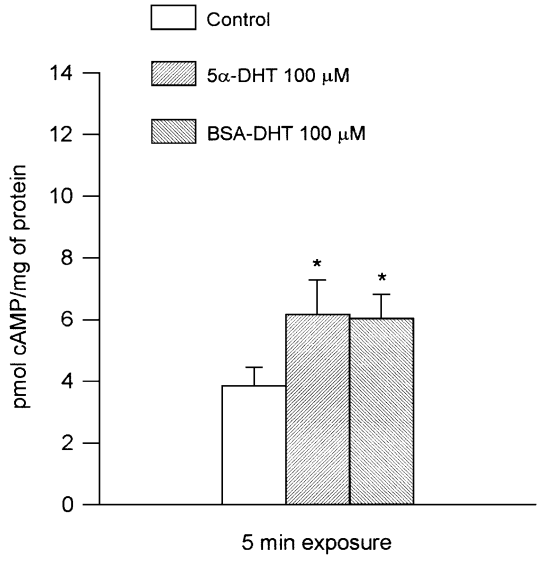

Fig. 5. Effect of 5 min of exposure to 5α-dihydrotestosterone (DHT, 100 μM) and 5α-dihydrotestosterone conjugated with BSA (BSA–DHT, 100 μM) on intracellular cAMP levels in electrically stimulated (0.5 Hz, 5 ms, 30–50% above threshold voltage) left atrium. cAMP levels are expressed as pmol/mg of protein. Each point represents the mean ± S.E.M. for at least six data. * $P<0.05$, by means of Student's *t*-test with respect to basal levels.

centage of contraction was smaller than that elicited by 5α-dihydrotestosterone. In addition, it significantly increased intracellular levels of cAMP (Fig. 5).

3.7. Effect of 5α- and 5β-dihydrotestosterone on [^{3}H]dihydroalprenolol binding to heart membranes of rat

To evaluate the possibility that androgen binding to β_1-adrenoceptors may be responsible for the cAMP increase and the positive inotropism, displacement experiments were performed using increasing concentrations of 5α- or 5β-dihydrotestosterone (0.1 to 100 μM) in the presence of the specific ligand, [^{3}H]dihydroalprenolol (1 nM). Binding of the radioligand, [^{3}H]dihydroalprenolol, to cardiac membranes was saturable (0.1 to 10 nM) and was displaced by propranolol. Under these experimental conditions, the Scatchard plot showed a receptor density of 84 fmol/mg of protein and a K_D of 0.85 nM (Fig. 6). The competition assayed with 5α- or 5β-dihydrotestosterone (0.1 to 100 μM) showed no displacement of the ligand with 5α-dihydrotestosterone. 5β-Dihydrotestosterone slightly decreased (24.9 ± 2.6% at 1 μM) the specific binding of [^{3}H]dihydroalprenolol (1 nM). There was no concentration-dependent displacement, and no significant differences existed between the different concentrations of androgens (Fig. 7).

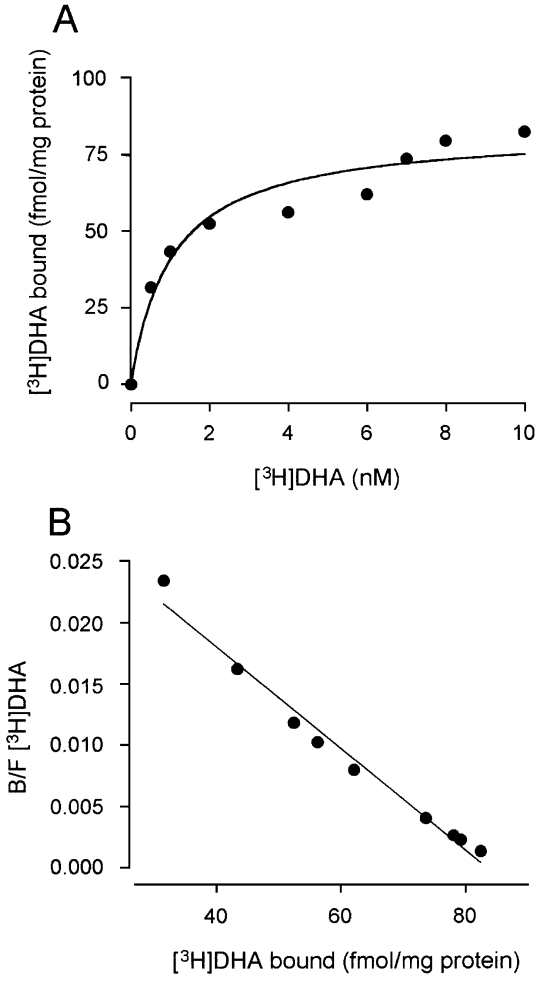

Fig. 6. (A) Specific binding of [^{3}H]dihydroalprenolol (DHA) to rat heart membranes as a function of the concentration of radioligand. The specific binding of radioligand was defined as the portion displaceable by propranolol (1 μM). All experiments were conducted in triplicate and repeated at least four independent times. (B) Scatchard plot obtained from same data. *B*: specific bound of radioligand. *B*/*F*: rate of specific bound and concentration of radioligand free.

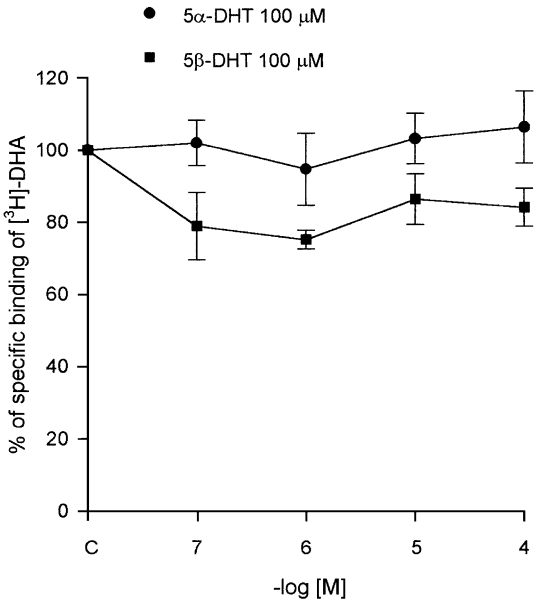

Fig. 7. Competition of specific [^{3}H]dihydroalprenolol (1 nM) binding to heart membranes by 5α- and 5β-dihydrotestosterone (DHT, 0.1 to 100 μM). Values represent the mean ± S.E.M. of five experiments.

3.8. Effect of 6 min exposure to 5α-dihydrotestosterone and isoproterenol on the expression of c-fos and c-jun in the left atrium of rats

To study the possibility of a rapid transcriptional effect on c-*fos* and c-*jun* in the left atrium of rats, the atria were exposed to single concentrations of 100 μM of 5α-dihydrotestosterone or 1 μM of isoproterenol for 6 min. Some atria were not exposed and were considered as controls. Then the tissues were removed from the bath, placed in liquid nitrogen and stored at −80 °C until used to determine the effect of these drugs on c-*fos* and c-*jun* mRNA levels.

Northern blotting analysis showed that acute exposure to 100 μM of 5α-dihydrotestosterone or 1 μM of isoproterenol did not modify the expression of c-*fos* and c-*jun* in the left atrium of the rat (data not shown).

4. Discussion

Our results confirmed the functional evidence that the androgens, 5α- and 5β-dihydrotestosterone, elicit positive

inotropism in the left atrium of the rat via cAMP-dependent mechanisms. Exposure to 5α- and 5β-dihydrotestosterone in the organ bath increased the intracellular levels of cAMP. Our findings showed that the basal levels of intracellular cAMP are consistent with those of previous studies of rat atria (Martínez et al., 1995). The levels of cAMP significantly increased 4 min after exposure to androgens and this increase was sustained, without significant changes, in the continuous presence of androgens in the organ bath. Therefore, it is highly likely that cAMP may be involved in the cardiotonic effect of the androgens. Similarly, the increase in contractility was also sustained. The time course of the cAMP increases was different from that elicited by isoproterenol, in which levels increase for at least 30 s after drug addition to the organ bath and then decrease in the continuous presence of the drug. The increase in inotropism is sustained. These kinetics are different from those observed in isolated cardiomyocytes, where the level of cAMP is maintained above basal levels in the presence of isoproterenol (Xiao et al., 1994). These differences may be related to the preparation used and not to a methodological unreliability of cAMP determinations, since cAMP levels were higher, up to 6 min, after exposure to the androgens. There is a delay in the time course of the increases in the intracellular cAMP elicited by androgens, but not in the contraction, where a latency period is not observed. This means that cAMP could be associated with the maintenance of contraction, whereas other membrane mechanisms may be involved in the establishment of the contraction (Koenig et al., 1989; García Valencia et al., 1992). In addition, as observed for the increase in positive inotropism, 5α-dihydrotestosterone seems to increase intracellular levels of cAMP in a concentration-dependent way. Incubation with IBMX, an inhibitor of phosphodiesterase, produced a synergistic effect on the 5α-dihydrotestosterone-induced increase in the intracellular levels of cAMP. It is unlikely that the effect of androgens is due to an inhibitory effect on phosphodiesterase.

The positive inotropism induced by the androgens in the rat left atrium was produced at a postsynaptic level, since the effect was elicited in reserpine-treated rats. Furthermore, under these experimental conditions, atenolol also antagonized the cardiotonic effect. This finding suggests a direct sympathomimetic effect of the androgens independent of the presynaptic release of catecholamines (Rubín et al., 1999). In fact, atenolol, besides antagonizing the contraction, inhibited the increase in cAMP levels elicited by 5α- and 5β-dihydrotestosterone. Furthermore, as observed with the contraction, there were no significant differences between the levels of cAMP in normal rats or those treated with reserpine. These findings led us to study the possibility of β-adrenoceptor activation, using specific binding assays for β-adrenoceptors. At the concentrations used 5α- and 5β-dihydrotestosterone caused a similar displacement of [^{3}H]dihydroalprenolol, the ligand for β-adrenoceptors, from the receptors. It seems that this effect is not selective or specific since a concentration-dependent relation does not exist.

These data support the hypothesis that androgens may increase the basal activity of unoccupied β-adrenoceptors (whose functional integrity is required) that could be coupled to adenylyl cyclase, resulting in an increase in intracellular cAMP. This possibility is very likely since an inhibitor of this enzyme, dideoxyadenosine, antagonized 5α- and 5β-dihydrotestosterone-induced contractions (Rubín et al., 1999). In this sense, it has been described that antagonists, besides inhibiting receptor-activated biological responses elicited by agonists, may also uncouple agonist-free receptors (Schutz and Freissmuth, 1992; Gotze and Jakobs, 1994).

Of great interest is the finding that 5α-dihydrotestosterone conjugated with BSA increased positive inotropism and cAMP levels. This suggests that an extracellular interaction of androgens may account for the cardiotonic effect.

Transcriptional regulation by cAMP is one of the best known mechanisms through which membrane receptors, via adenylyl cyclase activation, modulate the expression of certain genes (Barka et al., 1986; Lucas and Granner, 1992; Kolb et al., 1993; Aronica et al., 1994). In rat myocardial cells, cAMP induces c-*fos* expression (Iwaki et al., 1990). However, the precise signalling mechanisms that link the occupancy of β-adrenoceptors with the induction of genes is not well known.

It has been reported that steroids, including androgens, may produce cAMP-dependent transcriptional effects. Thus, estradiol via cAMP activates the cAMP-response element binding protein (CREB) that mediates the transcription of specific genes in breast cancer cells and uterus (Aronica et al., 1994). Here, we have confirmed previous pharmacological findings of rapid interactions between androgens and the plasma membrane causing cAMP-dependent mechanisms. These mechanisms could play a functional role in the enhancement of the contractility elicited by androgens, isoproterenol and forskolin, since the contractions were inhibited by actinomycin D and cycloheximide (Rubín et al., 1999). The presumable activation of transcription should be rapid in onset, suggesting that intracellular messengers may stimulate gene expression by modulating the activity of existing nuclear factors (Sasaki et al., 1984; Lewis et al., 1987; Spelsberg et al., 1991), as described in other preparations (Barka et al., 1986; Parker Botelo et al., 1988). The fact that β-adrenoceptor stimulation regulates a rapid and transient expression of c-*fos* and c-*jun* and that the polyamines are related to the modulation of c-*jun* expression (Wang et al., 1993; Patel and Wang, 1997; Bjersing et al., 1997) led us to study the role of these genes in the rapid positive inotropism elicited by androgens in the rat left atrium. Northern blotting analysis showed that, as described, in the rat heart the basal expression of c-*fos* is low in comparison to other tissues (Olson and Pessin, 1994). However, the early response genes, c-*fos* and c-*jun*, may be inducible with a short latency by different stimuli and act as positive regulators of cardiac transcription mitogens or growth factors (Mulroney et al.,

1996). However, neither 5α-dihydrotestosterone nor isoproterenol modified the expression of c-*fos* and c-*jun* at the same time (6 min) that they elicited an increase in the intracellular levels of cAMP and facilitated the contractility of the rat left atrium.

Therefore, androgens may increase intracellular cAMP. Furthermore, cAMP-dependent mechanisms could be related to the maintenance of the positive inotropism elicited by androgens in the isolated left atrium of the rat.

Acknowledgements

We thank Dr. C. López Otín (Universidad de Oviedo, Spain) for kindly providing us with the probe for c-*fos* and c-*jun*. This work was supported by grants from the Ministerio de Educación y Ciencia, Comisión Interministerial de Ciencia y Tecnología (DGES, PB95-1058 and PB98-1562), Spain.

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