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Testosterone and dihydrotestosterone inhibit gallbladder motility through multiple signalling pathways

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ABSTRACT

Testosterone (T) has been shown to cause vasodilation in rabbit coronary arteries through a nongenomic pathway. Part of this T-induced relaxation was shown to be mediated by opening voltage dependent K^+ channels. T infusion also reduces peripheral resistance in human males with heart failure. The effects of T or its active metabolite 5-α dihydrotestosterone (DHT) are not well studied. This study investigates the effect of T and DHT on contraction in guinea pig gallbladder strips. T or DHT induced a concentration-dependent relaxation of cholecystokinin octapeptide (CCK)-induced tension. Pretreatment of the strips with PKA inhibitor 14–22 amide myristolated had no significant effect on the relaxation induced by either T or DHT. Pretreatment of strips with 2-APB, an inhibitor of IP_3 induced Ca^{2+} release, produced a significant ($p < 0.001$) reduction in the T- or DHT-induced relaxation. Bisindolylmaleimide IV and chelerythrine Cl^- when used in combination had no significant effect on the amount of CCK-induced tension, but significantly ($p < 0.01$) decreased the amount of T- or DHT-induced relaxation. The flavone chrysin, an aromatase inhibitor, and genistein, an isoflavone, each produced a significant ($p < 0.01$) reduction in CCK-induced tension. Chrysin significantly ($p < 0.05$) increased T-induced relaxation; however, genistein had no effect on T-induced relaxation. It is concluded that T and DHT inhibits gallbladder motility rapidly by nongenomic actions of the hormones. Multiple pathways that include inhibition of intracellular Ca^{2+} release, inhibition of extracellular Ca^{2+} entry, and the actions of PKC may mediate this effect.

1. Introduction

Androgens have been shown to have nongenomic actions that are rapid and are mediated by interaction with steroid receptors or G protein-coupled receptors within the plasma membrane. The active metabolites of testosterone (T) include a 5α-reduced steroid, primarily dihydrotestosterone (DHT) that mediates many androgen actions in target tissues, including human myometrium [1].

T decreased myocardial ischemia in men with coronary artery disease by causing coronary vasodilation. The T-induced vasodilation was shown to be independent of the vascular endothelium and independent of nuclear androgen receptors, but was dependent upon receptors on the smooth muscle membrane with contained T binding sites. It was suggested that T directly inhibited both voltage gated Ca^{2+} channels and store-operated Ca^{2+} [2]. Supraphysiological concentrations (100 μM) of testosterone propionate caused a rapid

Abbreviations: ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; BK_{Ca}, high-conductance calcium-activated potassium channels; CCK, cholecystokinin octapeptide; DHT, 5α-dihydrotestosterone; P, progesterone; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; T, testosterone; TM, tropomyosin.

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vasorelaxation of the human radial artery in vitro. This T-induced vasorelaxation was mediated by increasing K^+ efflux through ATP-sensitive potassium channels (K_{ATP}), but not high-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) or voltage sensitive K^+ channels [3].

Epidemiological data suggest a role for sex steroids in the etiology of some airway diseases, in particular asthma. Sex steroids may have a direct effect on airway function and development. T relaxed precontracted tracheal smooth muscle. This effect required intact epithelium and was mediated by a nongenomic pathway and NO production [4].

This laboratory demonstrated that progesterone (P) affected the contractility of male guinea pig gallbladder strips in vitro. The relaxation of CCK-induced tension in the guinea pig gallbladder strips was concentration-dependent. The P-induced relaxation was shown to be mediated by multiple signalling pathways which included PKA/cAMP and inhibiting tyrosine kinase phosphorylation [5].

Since P was shown to relax tension in male guinea pig gallbladder strips, and T or DHT have been shown to affect the contractility of vascular smooth muscle and human myometrial smooth muscle; the purpose of this study was to determine if either androgen, testosterone (T) or its active metabolite, 5α-dihydrotestosterone (DHT), had any effect on the contractility of male guinea pig gallbladder strips.

2. Methods

Male Hartley guinea pigs (200–375 g body weight) were killed by cervical dislocation. The gallbladder was removed, cleaned, and placed in Krebs–Henseleit solution (KHS) that was gassed with 95% O_2 and 5% CO_2. The composition of the KHS was (mM) NaCl, 115; KCl, 5: $CaCl_2$, 2.1; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11. Each gallbladder was cut into strips (1.5 cm × 0.5 cm) and maintained in Sawyer–Bartlestone chambers filled with KHS, maintained at 37 °C, and gassed with 95% O_2 and 5% CO_2. An optimum resting tension of 0.7 g was determined previously and used in the study [6].

The force developed by the gallbladder strips was measured with Grass FT03 force displacement transducers and recorded on a Grass 5D polygraph (Grass Instruments Co., Quincy, MA). Isolated strips were equilibrated in the chambers for 45 min prior to determining their suitability for use. Each chamber had 2 μM atropine added, in every experiment, 3 min prior to 1.0 nM cholecystokinin octopeptide (CCK). The tension was measured. This was followed by three changes of KHS. The test was repeated twice with 25 min between tests. A repeatable minimum tension of 0.5 g had to be generated by the strips before use. All agents used were added directly to the chambers. All agents used to block a pathway which might mediate the T- or DHT-induced relaxation were added 3 min prior to the CCK unless otherwise noted.

Several series of experiments were performed to examine the effects of T or DHT on gallbladder strips. CCK (1 nM) was found to produce a stable long lasting tension after 3 min and was used throughout the study. This steady tension lasted at least 10 min [6]. In order to determine if T or DHT could relax CCK-induced tension, a concentration response curve was generated. The CCK-induced tension was allowed to reach a steady level (4 min, a standard time used throughout the study). The strips were exposed to one concentration of T or DHT, the response was observed until the relaxation reached a steady level (approximately 4 min), the KHS changed three times and the strips allowed to recover for 30 min before testing a different concentration of T or DHT. The concentration (50 μM) of T or DHT was selected for use in all the subsequent experiments as it produced a reproducible relaxation.

KCl (60 mM) was added to the chambers to induce tension. When the tension reached a steady level T was added to the chambers and the amount of relaxation in the tension was recorded. The chambers had the KHS changed three times and the strips were allowed for 30 min before the strips were used again.

In order to determine if T inhibited the tension induced by CCK, the strips were pretreated with T for 3 min prior to the addition of CCK. This was repeated using 1 μM nifedipine instead of T. The nifedipine was added to the chambers 3 min prior to the CCK.

Nitric oxide might mediate the relaxation induced by T. N^G-nitro-L-arginine methyl ester (L-NAME, 50 μM) was added to the chambers. When the CCK-induced tension reached a steady level, T was added to the chamber. Glibenclamide (20 μM), a blocker of ATP-sensitive K^+ channels, was also used in the same manner as L-NAME. Iberiotoxin (IBX; 50 μM), an inhibitor of BK_{Ca} channels was used to determine if the relaxation effect of T or DHT was mediated through K^+ channels.

In order to determine if the release of Ca^{2+} from the endoplasmic reticulum mediated the T- or DHT-induced relaxation 2-APB, a cell permeable inhibitor of IP_3-induced Ca^{2+} release, was added to chambers filled with normal KHS. 2-APB was added to the chambers 10 min prior to the CCK.

When PKA inhibitor 14–22 amide myristolated (PKA-IM; 180 nM) was used, it was added to the chambers 15 min prior to the CCK to ensure adequate time for entry into the smooth muscle. The effect of PKA-IM on both T- or DHT-induced relaxation was observed.

KT5823 (1.2 μM), a selective inhibitor of PKG, genistein (GEN; 10 μM), an inhibitor of protein tyrosine kinases and possible aromatase inhibitor, and chrysin (21 μM), an aromatase inhibitor, were used individually. The PKC inhibitors calphostin C (0.1 μM), chelerythrine Cl^- (5 μM), or the combination of bisindolymaleimide IV (0.5 μM) and chelerythrine Cl^- (5 μM) were used to determine the effects of PKC on either the T- or DHT-induced relaxation. These agents were added to the chambers 15 min prior to the CCK.

In order to determine if T affected cAMP levels, strips were prepared and suspended in the chambers and treated with CCK as in the tension studies. All of the strips were treated with CCK, the tension developed, and half of the strips were removed from the chambers, immediately frozen in liquid N_2 and kept at −80 °C until analyzed. The other half of the strips were treated in a similar fashion, except when the CCK-induced tension reached a steady level, T (50 μM) was added to the chambers. When the relaxation reached a steady level the strips were removed from the chambers, immediately frozen in liquid N_2 and stored at −80 °C until analyzed. An enzyme immunoassay kit for the direct measurement of cAMP was obtained from Assay Designs, Inc. (Ann Arbor, MI) and used to measure cAMP in the two groups of strips.