Journal of Andrology, Vol 17, Issue 5 502-508, Copyright © 1996 by The American Society of Andrology

JOURNAL ARTICLE

Purification and characterization of a lipophilic factor from testicular macrophages that stimulates testosterone production by Leydig cells

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Testicular macrophages have been shown to secrete a factor that stimulates testosterone production by Leydig cells. The purpose of this investigation was to purify and characterize this factor. Medium was collected from 24- to 48-hour cultures of testicular macrophages isolated from adult rats. This medium induced a sevenfold increase in testosterone production by cultured Leydig cells. When the medium was extracted with ether, all biological activity was found in the organic phase, indicating that the factor was lipophilic. The ether extract was then fractionated on a C18 reversed-phase high-performance liquid chromatography (HPLC) column, using a gradient of acidified methanol as the mobile phase. Leydig cell-stimulating activity eluted at approximately 11 minutes. Standards of testosterone, dihydroepiandrosterone (DHEA), pregnenolone, progesterone, dihydrotestosterone (DHT), and prostaglandin E2 (PGE2) all had elution times of between 5 and 6 minutes, under identical column conditions. The biological activity of the HPLC-purified fraction was partly resistant to boiling but was completely abolished by Dextran-coated charcoal treatment. Biological activity of testicular macrophage-conditioned medium was not abolished following chymotrypsin treatment, indicating that this molecule was not a hydrophilic peptide. It was found that the factor obtained by reversed-phase HPLC could be further purified by normal-phase HPLC. The results of this investigation demonstrate that the testicular macrophage-derived factor that stimulates testosterone production by Leydig cells can be purified by organic extraction and HPLC, and that it is a highly potent chymotrypsin-resistant heat-stable lipophilic factor.

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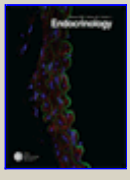

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