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JOURNAL ARTICLE

Characterization of functional Leydig cells after purification on a continuous gradient of percoll

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Two human chorionic gonadotropin (hCG) responsive cells from rat testicular interstitium were previously isolated on a discontinuous gradient of Percoll. The light cells were non-steroidogenic and bound 125I-labeled hCG with high affinity (Kd 3.0 x 10(-10) mol/L), whereas the steroidogenic heavier cells (Leydig cells) produced cyclic adenosine monophosphate (cAMP) and testosterone in response to hCG stimulation with very little hCG binding. In that study, the heavier cell fraction was contaminated with germ cells, red blood cells, and other cells. These cells have now been further purified on a continuous gradient of Percoll (20 to 60%, v/v), and have resolved into three visible bands. The cells in subfraction I, predominantly damaged Leydig cells, germ cells, and/or residual light cells, bind 125I-labeled hCG with high affinity (Kd 4.09 x 10(-10) mol/L) without producing cAMP and testosterone in response to hCG. Subfraction III consists mainly of red blood cells. The cells in subfraction II, identified as typical Leydig cells by electron microscopy, produce cAMP and testosterone in response to hCG but, again, bind only a small amount of hCG (4.5 +/- 0.3 fmol/2 x 10 (6) cells/250 microliters/per hour at 37 degrees C). Thus, further purification of the heavier cell fraction from a discontinuous gradient of Percoll on a continuous gradient of Percoll yields Leydig cells, free of contaminating germ cells and red blood cells, which actively produce cAMP and testosterone with a very low level of hCG binding, the affinity of which is undetectable by current binding techniques.

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