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Calcium- and glucose- dependent pulsatility of LHRH release from the rat hypothalamus in vitro.

We have recently shown the pulsatile pattern of LHRH release in vitro from the retrochiasmatic hypothalamus (RCH) of male rats and the increased frequency of these LHRH pulses before puberty (Endocrinology, May 1984). Additional experiments were carried out to evaluate the specificity of the in vitro observations. Single, unagitated RCH removed from pubertal rats were immediately incubated in 0.5 ml Dulbecco's Modified Eagle's Medium (DMEM) renewed every 7.5-min period. In these serial samples, the release of LHRH was determined using a highly sensitive RIA. A significant pulse was ≥ 6 pg/7.5 min (4 variation coefficients of the recovery of synthetic LHRH). In control conditions (DMEM with 1.25 mM Ca^{++} and 25 mM glucose), a depolarization by 20 or 50 µM veratridine (n=69) induced a marked increase ($p < 0.001$) in LHRH release (19.8 pg/7.5 min). The mean response to veratridine was significantly lowered to 5.7, 1.5 and 2.8 pg/7.5 min, in the presence of 5.6 mM 2-deoxyglucose (n=28), 20 mM EGTA (n=10) and 0.1 mM D-600, a calcium channel blocker (n=11) respectively. In control conditions, the mean frequency of spontaneous LHRH pulses was 2.8/2h. A significantly reduced frequency (1.1, 0.2 and 0.3 pulses/2 h) was observed with 2-deoxyglucose, EGTA and D-600. In conclusion, the availability of Ca^{++} and, to a lesser extent, glucose, may determine the occurrence of pulses in LHRH release from the RCH, either spontaneously or induced by a depolarization, in vitro.

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Polyclonal versus monoclonal antibodies : application to the study of human anti-Müllerian hormone.

Monoclonal antibodies, raised against partially purified AMH, have allowed the purification of the hormone to homogeneity, and the development of radioimmunological and immunocytochemical methods, which could however be applied only to bovine AMH (bAMH), because of the zoospecificity of the first generation of monoclonal antibodies. Wishing to use these methods in clinical studies, we have sought to obtain antibodies recognizing human AMH. Two methods have been used. A second generation of monoclonal antibodies was obtained by immunization with pure bAMH. Thirteen clones, screened through an immunodot procedure using bAMH as antigen, have been identified. Their zoospecificity has been tested by using them as first antibody in an immunocytochemical reaction performed on testicular sections of different species. Caprine and ovine AMH are recognized by all monoclonal antibodies reacting with bAMH, most of these are IgG clones with high affinity. Three IgM clones recognize pig AMH, and one binds to human AMH. In parallel, a polyclonal antibody has been raised in a rabbit immunized with pure bovine AMH. This antibody is not zoospecific, and has allowed an immunocytochemical study of AMH in human testicular tissue, through an avidin-biotin-immunoperoxidase method, performed upon cryostat sections of testicular biopsies or autopsy material. AMH has been identified in the Sertoli cells of 6 human fetuses, aged 16 to 22 weeks, and 4 boys aged 1 month to 5 years. This method can be used to study testicular AMH in intersex disorders.

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Comparison of the immunochemical, receptor binding and biological properties of natural insulin-like growth factor I/Somatomedin C (IGF-I/Sm C) and its recombinant DNA-synthesized Thr-59 analog.

We have synthesized a gene coding for an analog of IGF-I/Sm C and cloned it into a plasmid for expression in E. coli. This analog, containing Thr-59 and 8 extra amino acids at the N-terminal, was purified to homogeneity and tested for various activities. The ^{125}I-labeled IGF-I analog has an affinity for Sm binding protein as well as antibodies raised against human-derived IGF-I that is slightly less than the natural molecule. Both ^{125}I-labeled IGF-I and its analog bind to a specific cell membrane receptor on cultured human fibroblasts (HF) and rat chondrocytes (RC) in a time-, temperature-, and concentration-dependent manner. The analog stimulates the incorporation of ^{3}H-thymidine into DNA in cultured RC and HF in a dose-response manner, with half-maximal stimulation at 12.5 and 62.5 ng/ml respectively, and is equipotent to natural IGF-I. In contrast, the Thr-59 analog did not enhance ^{35}S-sulfate incorporation into chondroitin sulfate in RC in a fashion similar to the natural hormone.

We conclude that this IGF-I analog has similar, though not identical, immunochemical, receptor binding and biological properties to human-derived IGF-I/Sm C. Nevertheless, we anticipate this peptide will be useful for studying the activity of this Sm.

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Elevated IGF II in cerebrospinal fluid and brain in a newborn with extreme megalencephaly.

Recent studies show that the insulin-like growth factor II (IGFII) in contrast to IGF I appears ineffective in stimulating longitudinal growth. Both factors are present in human serum, whereas in CSF only IGF II can be detected. This hormone has been suggested to be produced in significant amounts in brain.-We report a patient with extreme megalencephaly with markedly elevated concentrations of IGF II in CSF and cerebral cortex. Head circumference at birth (gestational age 34 weeks)was 40.5cm (+4.8SD); it increased to 51.5cm (+8.9SD) at the age of 4 1/2 months when the boy died. He had muscular hypotonia and severe epilepsy. Body length (including head) was on the 97th percentile. The cause of megalencephaly could not be identified. IGF I and II in serum were in the normal range for age. IGF II in CSF was 24 ng/ml compared with 10 ng/ml in a pool of CSF from controls of the same age. At autopsy brain weight was 1450 g (normal range 567±81g). IGF II extracted from frontal cortex was 50ng/g compared to 3-11 ng/g in four controls.

We speculate that the megalencephaly of this patient has been caused by an excess of IGF II in brain. This data supports the assumption that IGF II is a major factor in CNS growth.

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Receptors for Insulin-like Growth Factor II on human fibroblast monolayers.

Two types of somatomedin/insulin-like growth factor (SM/IGF) receptors have been defined. Type I receptors have a higher affinity for SM-C/IGF than IGF-II and bind insulin at high concentrations. Type II receptors have a higher affinity for IGF-II than SM-C/IGF I , and do not bind insulin. In this study we have characterized the binding ofIGF-II to human monolayer fibroblast cultures. ^{125}I-IGF-II binds to the human fibroblasts with anaverage specific binding of 12,5% /2oo,ooo cells. The binding of IGF-I was 25% and of insulin was 4,5% of the specific IGF-II binding. The IGF-II was bound to a high affinity receptor. Half maximal displacement was seen with 12 ng/ml IGF-II and 45 ng/ml IGF-I. Insulin at high concentration caused no displacement. The estimated affinity of the IGF-II receptor was 1,2 nM with calculated 4oo,ooo receptor sites/cell. No autoregulation of these IGF-II receptors was seen by homologous hormone. Serum deprivation led to a 5o% increase in IGF-II receptor sites. IGF-II binding to human fibroblasts fits the criteria for a type II receptor. There are more receptors for IGF-II on these cells than for either IGF-I or insulin. This suggests an important metabolic role for IGF-II in normal human fibroblasts.

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Serum-growth-promoting activity in human newborns: relationship of thymidine activity with birth weight and the length of gestation.

Thymidine Activity (TA) was measured by the effect of serum upon incorporation of 3H-thymidine into lectin-activated lymphocytes in 54 newborns (NB): 32 full-term, 11 preterm and 11 with intrauterine growth retardation (IGR). Capillary blood was collected at 0-4 hr, with routine samplings. TA values were lower in preterm (.476 ± .079 U/ml) than in IRG (.91 ± .118 U/ml, $p<.01$) and in full-term NB (1.237 ± .06, $p<.001$), and also in IGR than in NB with normal weight ($p<.025$). In all the NB studied, TA was significantly correlated with gestational age ($r = .662$, $p<.001$) and birth weight ($r = .546$, $p<.001$). Longitudinal studies up to 21 days did not show significant changes in full-term NB, while in preterm and IGR infants TA increased progressively to reach normal values at the 21th day. In conclusion, the correlations observed in newborns between TA, birth weight and gestational age, and the postnatal normalization in infants with low birth weight, show that TA directly reflects the nutritional state of the fetus.