Hormones

BIT 230
Walsh Chapter 8
Hormones

- Regulatory molecules
- Affect all areas of metabolism
- Endocrine- hormones travel via the bloodstream to its target cell: “true hormone”
- Modern definition- any regulatory substance carries a cell signal elsewhere in the body (includes autocrine and paracrine signals)
True Endocrine Hormones

- Insulin
- Glucagon
- Growth Hormone
- Gonadotrophins
Insulin

- Large part of chapter devoted to insulin
- We have covered insulin a lot in program, but some slides of some new material
- Produced in β cells of pancreatic islets
- Regulates blood glucose levels, in narrow limits: 3.5-8.0 mmol/L (regardless of food intake)
- Some mitogenic properties at high levels (likely via IGF-1 receptor)
Insulin cont’d

- Target cells are skeletal muscle fibers, hepatocytes and adipocytes

- In these cells, antagonize effects of other hormones (glucagon)

- Insulin release after increase of blood glucose levels
Insulin cont’d

- Stimulates glucose transport
- Stimulates other biosynthetic (anabolic) pathways (converts nutrients to storage forms)
- Inhibits catabolic pathways (where molecules are broken down)
- Stimulates protein and DNA synthesis (insulin’s growth properties)
IDDM – insulin dependent diabetes mellitus (Type 1 or juvenile onset)

Failure of body to synthesize insulin in sufficient quantities

T-cell mediated autoimmune destruction of pancreatic islet cells (in predisposed people)

Controlled by s.c. injection of insulin
Insulin cont’d

- Insulin receptor: large glycoprotein with 2-735 aa alpha chains and 2-620 aa beta chains (see Figure 8.2 page 308)

- Animal preps of insulin contain some immunogenic contaminants (e.g., glucagon) that react in humans

- Before rInsulin, many purification steps needed before use in humans (various chromatography steps)
Recombinant Human Insulin

- First approved in 1982 (USA, West Germany, UK and Netherlands)
- First recombinant product for therapeutic use in humans
- Advantages:
  - Supply ensured
  - Economical
  - Less immunogenic (except remove bacterial cell contaminants)
  - no risk of transmission of disease from the animal
Recombinant Insulin cont’d

- Pancreas from 1 pig – enough insulin for 1 diabetic for 3 days
- Recombinant allows for endless supply
- Genentech and Lilly first to produce
- Genentech expressed α and β chains in separate cells then mixed
- Eli Lilly expressed proinsulin, followed by cleavage of mature insulin
Table 8.3, page 315 shows different companies that produce insulin.

Same page Figure 8.6 purification scheme for insulin (see next slide).

Can be quick acting formula (s.c injection) or slowing acting, but longer duration of action (also by injection).
Purification Scheme for Insulin

Figure 8.6. A likely purification scheme for human insulin prb. A final RP-HPLC polishing step yields a highly pure product. Refer to text for details.
Therapeutic Insulin

- Normal insulin up- or down regulated all day in response to blood glucose
- Can’t mimic this process exactly in diabetics
- Inject slow and/or fast acting insulins to regulate blood sugar
- Patients administer insulin before meals and plan their injects carefully
Therapeutic Insulin cont’d

- To stabilize insulin:
  - add zinc - promotes zinc-insulin crystals, take longer to dissociate and leak from injection site, so remains in blood longer
  - Addition of a protein – insulin will complex with protein, causing slow release of insulin from the complex. Use protamimes, basic proteins
Pharmacokinetic properties of Insulin

- rDNA technology allows for modified amino acids in insulin
- Why do this?
  - Identification of insulins with altered pharmacokinetic properties (slow or fast acting)
  - Identification of “super-potent” insulins-higher receptor affinity (may lead to lower dose requirements)
Examples

- AA residues of insulin which bind receptor: A1, A5, A19, A21, B10, B16, B23-25
- Change for histidine to glutamate at B10 shows 5-fold greater activity in vitro
- Not always same effect in vivo, but try out the substitutions
- Insulin Lispro- fast acting analogue-
Human Insulin vs. LisPro
Taken from http://www.aafp.org/afp/980115ap/noble.html
Glucagon

- 29 aa single-chain peptide (3500 Da)
- Synthesized by α cells of pancreas
- Made as a proform and cleaved
- Opposes action of insulin
- Stimulates breakdown of glycogen, lipid and protein
- Increase blood sugar levels (after long period without food)
Glucagon cont’d

- Cell surface receptor mediated
- cAMP signaling pathway
- Acts to prevent hypoglycemia (frequent complication of insulin administration in diabetics), due to:
  - Giving too much insulin
  - Giving insulin too long before a meal
  - Increased physical activity
  - Treated with glucose, but can be treated by glucagon
Uses of Glucagon

- Diagnostic during stomach radiological exams – inhibits motility of the intestinal track
- Glucagon traditionally prepared from bovine or porcine preparations (human, pig and cow identical molecules)
- GlucaGen- trade name of recombinant product (Novo Nordisk)
Growth Hormone (GH)

Talked about it in chapter 7 - under growth factors; largely skip in this chapter (1-2 slides only)

- Biological effects of GH:
  - Increased bone and skeletal muscle growth
  - Protein synthesis in tissues
  - Elevation of blood glucose levels
  - Enhanced renal function
  - Increase of muscle and cardiac glycogen stores
Some companies that make GH – Lilly, Genentech, Serono, Novo Nordisk

Therapeutic uses:
- Turner syndrome (female with one X)
- Idiopathic short stature - main clinical use
- Chronic renal failure
- Induction of lactation
- Body building
- Induction of ovulation
Gonadotrophins

- Gonads primary target tissues
- Reproductive function and development of secondary sex characteristics
- Synthesized by the pituitary
- FSH- follicle stimulating hormone
- LH- luteinizing hormone
- hCG- human chorionic gonadotrophin
<table>
<thead>
<tr>
<th><strong>Sex Hormones</strong></th>
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<tbody>
<tr>
<td><strong>FSH</strong></td>
</tr>
<tr>
<td>34 kDa</td>
</tr>
<tr>
<td>Chrom. 6 (α)</td>
</tr>
<tr>
<td>Chrom. 11 (β)</td>
</tr>
<tr>
<td>made in pituitary</td>
</tr>
<tr>
<td>Follicular growth (females)</td>
</tr>
<tr>
<td>Spermatogenesis (males)</td>
</tr>
<tr>
<td><strong>LH</strong></td>
</tr>
<tr>
<td>28.5 kDa</td>
</tr>
<tr>
<td>Chrom. 19</td>
</tr>
<tr>
<td>made in pituitary</td>
</tr>
<tr>
<td>Induction of ovulation (female)</td>
</tr>
<tr>
<td>Testosterone (males)</td>
</tr>
<tr>
<td><strong>CG</strong></td>
</tr>
<tr>
<td>produced in pregnancy</td>
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<tr>
<td>Maintenance of developing embryo</td>
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</table>
Sex Hormones

- Exert their effects via (you guessed it!) cell surface receptors (GPCRs- G-protein coupled receptors)

- Signal continues via adenylate cyclase and the release of cAMP
Therapeutic Roles for Gonadotrophins

- Treating infertility (also can be “sub-fertility”) - Serono, Organon makes these

- Late 90’s a $250 million market (small by drug company standards but important)

- Can’t get human pituitary to extract hormones, use post-menopausal human urine until recombinant sources available
Steroid Hormones

- Estrogen and Progesterone
- Birth control pills
- Some recent controversy with these
- Wyeth big maker
- Peri- and during menopausal symptoms
  - Hot flashes
  - Night sweats
TSH/Calcitonin

TSH
- Thyroid stimulating hormone
- Member of gonadotrophin hormones, but targets thyroid gland
- Diagnostic aid in thyroid cancer

Calcitonin
- Regulated serums calcium and phosphate levels
- Treat hypocalcaemia with Paget’s disease and malignancy