NERNST POTENTIAL PRACTICAL

Membranes and Membrane Potentials	**Resting Membrane Potential** → all cells have a resting membrane potential, which is essential to their normal function
	Ringer's solution → contains ions essential for cell function. One litre of lactated Ringer's solution contains: • 130 mEq of *sodium* ion = 130 mmol/L • 109 mEq of *chloride* ion = 109 mmol/L • 28 mEq of *lactate* = 28 mmol/L • 4 mEq of *potassium* ion = 4 mmol/L • 3 mEq of *calcium* ion = 1.5 mmol/L
	Ion channels → voltage-dependent, membrane-imbedded proteins that pass a particular ion, usually selectively, in one direction (dependent on the electrical and chemical gradients) or can be activated by a ligand • Ca^{2+} channels (L-type and T-type) • K^{+} channels (at least 15 types in heart) • Na^{+} channels (one major type)
	Pumps → ATP-dependent and voltage-independent membrane-imbedded proteins responsible for the movement of ions against a chemical gradient (and possibly an electrical gradient)
	Exchangers → voltage-independent & ATP-independent membrane-imbedded proteins that pass usually two different species of ions, uni- or bi-directionally (affected by chemical and electrical gradients)
Channel Activation	**Na+ channel** → S4 region of the channel contains highly charged amino acids, and physically moves in response to voltage change (depolarisation); the voltage change causes the amino acids to rearrange, and the channel to open.
Voltage-activated Ion Channels	Ion channels can be in three different states (or transitions) which must occur in the order that follows: i) Reprimed ii) Activated iii) Inactivated: channel stops passing current, even with maintained depolarisation The channels movement through the phases is largely voltage-dependent, but in many cases is also time-dependent (when moving from activated to inactivated)
Pumps	Pumps exist to maintain required gradients; pumps work against concentration gradients (e.g.- Na+/K+ pump or Ca2+ pumps) and thus require energy, by splitting ATP, to move ions against it.

Exchangers	Exchangers also move ions against its gradient, but instead of using energy from ATP to do this, it uses a *favourable gradient* of another ion.
Membrane and Action Potentials	**Membrane Potential** → determined by the sum of potassium/sodium channels that are open
	Potassium Conductance → the amount of potassium ions flowing through the channel; increase in potassium conductance causes drop in membrane potential, and maintains resting potential
	Sodium Conductance → the amount of sodium ions flowing through the channel; sharp increase in sodium conductance causes a spike in membrane potential, known as an **action potential**
Nernst Potential	**Nernst Potential** → the membrane potential at which there is no net (overall) flow of that particular ion from one side of the membrane to the other Nernst Potential = (RT/F) * ln($\frac{[K+]out}{[K+]in}$) • K^+ will leak out of the cell along its **chemical concentration gradient** - This results in an **electrical gradient** across the membrane - As increasing amounts of K^+ leak out of the cell, the electrical gradient increases • Because opposites attract, the increasingly negative inside of the cell can exert a pull on the positively charged K^+ - Thus, the electric gradient grows to oppose the chemical gradient until an **equilibrium** is reached - The membrane potential at which this equilibrium is reached is the **Nernst Potential**
Goldman-Hodgkin-Katz Equation	Nernst Potential = (RT/F) * ln($\frac{PNa[Na+]out+PK[K+]out+PCa[Ca2+]out}{PNa[Na+]in+PK[K+]in+PCa[Ca2+]in}$) • The Goldman Hodgkin Katz equation is a more accurate representation of a living cell - It accounts for **permeability** (P) and contribution of **all ions** that can cross the membrane • The membrane potential is determined by the ion with the greatest permeability

ENDOCRINOLOGY

<table>
<tr><td colspan="2">Endocrinology → study of hormones and their actions</td></tr>
<tr><td colspan="2">Endocrine glands → produce cell signalling molecules called hormones</td></tr>
<tr><td colspan="2">Hormones → associate with a receptor to induce a physiological change within a target cell</td></tr>
<tr><td rowspan="3">Physiological Responses to Hormones</td><td>Response to a hormone → depends on concentration of free, biologically active hormone</td></tr>
<tr><td>Concentration of the free, biologically active hormone → depends on:

• Secretion rate from the endocrine gland
• Half-life of the hormone and how it is metabolised
- Some hormones need metabolism to active them
- Others are excreted by metabolism
• Whether it is bound to plasma proteins while in circulation
- Bound hormones are not free, biologically active hormones</td></tr>
<tr><td>The response to a hormone depends on →

• Sensitivity of target cells
• Presence of receptors
• Activation of receptors (leads to activation of second messengers)
• Intracellular signal transduction pathway (which brings about physiological change)</td></tr>
<tr><td rowspan="2">Endocrine Diseases</td><td>Too much hormone activity → caused by hypersecretion, reduced plasma protein binding, reduced clearance/metabolism, excessive response at target tissue (rare)</td></tr>
<tr><td>Too little hormone activity → caused by hyposecretion, increased clearance/metabolism, tissue resistance or insensitivity (common)</td></tr>
<tr><td rowspan="2">The Hypothalamus</td><td>Structure of the Hypothalamus → located at the base of the brain; connected to the pituitary gland by the connecting stalk

• Made up of nuclei (concentrations of neuronal cell bodies) and nerve tracts (axons)

• Regulates and coordinates endocrine responses to changes in the external and internal environment</td></tr>
<tr><td>Function of the Hypothalamus → include:

• Regulation of behaviour
- Reproductive, feeding, rage, fear

• Regulation of homeostasis
- Body temperature, metabolism, water balance (blood volume, thirst, urine output), growth, stress, reproduction

• Often regulates a combination of physiology with behaviours
- E.g.- regulates water balance by promoting thirst</td></tr>
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Hypothalamus integrates stimuli from both the external and internal environment and produces an appropriate output

Receives neural input from other brain centres to effect endocrine output
Receives humoral (fluid-borne) input, signals (hormonal, metabolites) that come via the blood and cross the blood-brain barrier and are then present in the cerebrospinal fluid
Outputs can be both neural and humoral

Endocrine output from the hypothalamus is really about the regulation of the pituitary gland

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<tr><td></td><td>Neurohormones → hormones synthesised within neurons</td></tr>
<tr><td>The Anterior Pituitary Gland</td><td>
Cell types of the Anterior Pituitary Gland → 5 different types that each produce their own hormone(s); each hormone effects their own endocrine gland or tissue:

Somatotrophs

Growth Hormone
Liver and other tissues

Lactotrophs

Prolactin
Breasts

Gonadotrophs

“Gonadotrophins”: LH (Luteinising Hormone) & FSH (Follicle Stimulating Hormone)
Gonads (ovary and testis)

Thyrotrophs

TSH (Thyroid Stimulating Hormone)
Thyroid gland

Corticotrophs

ACTH (Adrenocorticotrophic Hormone)
Adrenal cortex

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Capillaries of the Anterior Pituitary Gland →

Fenestrated Capillary

‘Fenestra’ is latin for window
More open capillary, many gaps in the endothelial cell membrane
Allows for the passage of small proteins (including hormones) between the blood and interstitial fluid
Capillaries in anterior pituitary are discontinuous

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Regulation of Anterior Pituitary Gland	**Hormone Secretion** → in response to **neurohormones** called "releasing hormones/factors" (or "hypophysiotropic factors") from the hypothalamus • Hypothalamus axons synapse at a capillary network, the **median eminence** - Neurohormones are released into the capillary network - Neurohormones travel down the pituitary stalk via the **hypothalamic-hypophyseal portal system** - Neurohormones bind to receptors in the anterior pituitary, stimulating the release of hormones - Hormones then travel in another bloodstream to their tissues • Regulation of the anterior pituitary gland by the hypothalamus via circulation - Releasing factors are often small peptides with a short half-life • Activity of each cell type is **regulated independently** of the others - Releasing factors that control each hormone • Hormones can be controlled by both Releasing and Releasing Inhibiting Factors, though usually one is dominant over the other - In hypothalamo-pituitary disconnection, most hormone secretion rates go down, because **Releasing Factor** primarily controls their secretions - In hypothalamo-pituitary disconnection, prolactin concentrations actually go up, because **Releasing Inhibiting Factor** primarily controls the secretion of prolactin
Growth Hormone	**Growth Hormone** → promotes muscle deposition and at the same time it promotes fat breakdown; previously called somatotrophin
	Human Growth Hormone (hGH) → 191 amino acid polypeptide, folded into a globular, anabolic protein
	Growth Hormone Concentrations in Circulation → growth hormone is released in a pulsatile manner over a 24-hour period • Pulses of growth hormone released during the day - Higher amplitude pulses of growth hormone released during sleep • In males, it is more typical to see high amplitude, infrequent pulses - In females, it is more typical to see smaller amplitude, frequent pulses • Tissue response to growth hormone depends on pulse **frequency** and pulse **amplitude** • The amount of growth hormone released declines with age, as does the amplitude and frequency of pulses - Peaks around puberty

Control of Growth Hormone Secretion →

- Growth Hormone Releasing Hormone
  - Neurohormone from the hypothalamus
  - Driving growth hormone secretion (and thus pulsatility)
  - 43 amino acid peptide

- Somatostatin
  - Releasing Inhibiting Factor
  - Inhibits secretion of growth hormone (and thus inhibits pulsatility)
  - 14/28 amino acid peptides

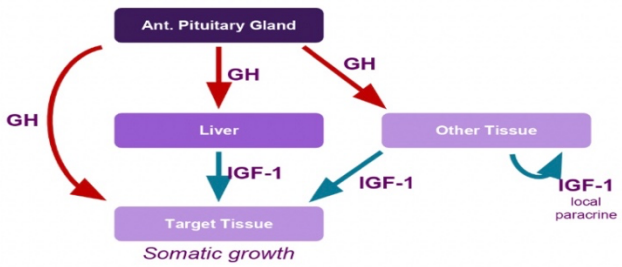
- Somatotroph (in anterior pituitary)
  - Has both GHRH and Somatostatin receptors
  - Both have **GPCR's**
  - GHRH binds and initiates the following pathway:
    - Stimulates the generation of cAMP within the somatotroph
    - This phosphorylates protein kinase A
    - This opens the voltage-gated calcium channels, resulting in calcium influx into the cell
    - Calcium influx causes vesicle fusion (vesicles are filled with growth hormone)
    - Growth hormone is then secreted from the somatotroph into peripheral circulation
  - Somatostatin, rather, inhibits cAMP, resulting in less PKA phosphorylation, calcium influx and hormone release
  - A convergence of signalling pathways

- Regulation via **short-loop negative feedback** to the hypothalamus
  - Growth hormone exerts negative feedback on both the GHRH neurons and the somatostatin neurons
  - High GH levels switches GHRH off and somatostatin on

Actions and Signalling of Growth Hormone →

- Major determinant of growth
  - In general, its actions are anabolic
  - Increases number and size of cells in soft tissues
  - Increases thickness and length of long bones
  - Also has some *metabolic actions* distinct from growth effects

- Acts in most cells of the body by binding to growth hormone receptors to activate signal transduction pathways

	- Receptors are dimers, growth hormone locks onto both arms of the dimer to activate it and the signal transduction pathway • Three major signal transduction pathways: - Janus Kinase Signal Transducer and Activation of Transcription (JAK/STAT) pathway - MAP Kinase (MAPK) pathway - PI3 Kinase AKT (PI3/AKT) pathway - In each pathway, the signalling ends up signalling through to the nucleus to regulate gene transcription ○ Many activate changes in transcription that drive growth
Growth Hormone and IGF-1	**Somatomedin Hypothesis** → growth hormone acting via IGF-1 drives tissue growth; growth hormone causes hepatocytes to release *IGF-1*, which travels to target tissues and drives somatic growth • Developed in the 1950's, and still holds true - Growth hormone, however, can act directly on target tissues to stimulate cell growth (doesn't have to work solely through IGF-1) - Some effects of growth hormone are direct, others are indirect (IGF-1) - Growth hormone can also act on *any cell* by stimulating IGF-1 production in those cells (IGF-1 then acts as a local paracrine) ○ IGF-1 not necessarily just from the liver 
	IGF-1 → Insulin-like Growth Factor 1; they are polypeptide hormones that have structural regions which share homology to insulin • IGF-1 is the major determinant of postnatal growth • *IGF-2* is thought to be important for in utero growth (also structurally related to insulin and IGF-1) - The receptors for both IGF-1 and IGF-2 show some structural similarities - Both receptors are *tyrosine kinase receptors*, meaning they automatically phosphorylate intracellularly when the hormone binds • IGF-1 Signalling Pathways - MAP Kinase Pathway - PI3 Kinase AKT Pathway - Similar pathways to growth hormone

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Pathways signal to the nucleus, and drive cell hypertrophy, cell hyperplasia or proliferation, and they can increase cell survival

The ways by which IGF-1 drives growth

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<tr><td rowspan="4">Post Natal Growth</td><td>Post Natal Growth → all growth after birth; cell hypertrophy (mostly) and cell hyperplasia</td></tr>
<tr><td>Hypertrophy → the enlargement of an organ or tissue from the increase in size of its cells (increase in cell size)</td></tr>
<tr><td>Hyperplasia → the enlargement of an organ or tissue caused by an increase in the reproduction rate of its cells (increase in cell number)</td></tr>
<tr><td>
Growth Velocity → high in the first two years of life (postnatal growth spurt) and around the onset of puberty (pubertal growth spurt)

Growth rates remain reasonably high across childhood, and drops significantly post-puberty and ceases by the early 20s
Growth hormone is the major hormone that drives postnatal growth

In childhood, there is high growth hormone pulsatility
Also drives pubertal growth spurt

Plasma concentrations of IGF-1 are low in postnatal years

Mainly direct growth hormone during early growth
IGF-1 concentrations increase across childhood, and max out around the age of puberty
IGF-1 does fuel the pubertal growth spurt

Over a 24-hour period, IGF-1 levels are fairly stable, despite its secretion being stimulated by pulsatile Growth Hormone

Synthesis of IGF-1 takes some time following the growth hormone pulse
IGF Binding Proteins maintain pool of circulating IGF-1, resulting in slower degradation (and thus stabilising IGF-1 levels in circulation)

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<tr><td>Actions of Growth Hormone</td><td>

Bone

Long bone growth driven by GH and IGF-1
Occurs at the ends of each long bone (epiphyseal plates)
Constant cell division and hypertrophy lead to an increase in bone length
GH acting directly increases cell proliferation
IGF-1 more responsible for cell hypertrophy

Other (metabolic) actions of growth hormone (via direct action):

Muscle

Stimulates amino acid uptake
Decreases glucose breakdown

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	○ Inhibits protein breakdown ○ Increases muscle mass by these processes - **Adipose Tissue** ○ Decreases glucose uptake ○ Increases fat breakdown (lipolysis) ○ Decreases in fat deposits by these processes - **Liver** ○ Increases protein synthesis ○ Increases gluconeogenesis (glucose output) ○ Overall increases blood glucose levels by these processes ○ Not that important usually, only in a starvation state -
Growth Hormone: Regulation & Integration	**Short loop negative feedback** → growth hormone acts in a short loop negative feedback to inhibit its releasing factor, GHRH, and promote its releasing inhibiting factor, somatostatin
	Long loop negative feedback → circulating IGF-1 can work in a negative feedback loop to control the release of GH at both the anterior pituitary and hypothalamus (by virtue of GHRH and somatostatin) levels
	Hypothalamus: An Integrative Centre → the hypothalamus controls growth (growth hormone pulsatility) via an integrated response to *hormonal*, *metabolic* and *neural* inputs • Hormonal Inputs - Sex steroids increase GH pulsatility • Metabolic (Humoral Inputs) - Metabolites such as high amino acid levels, low fatty acid levels, low glucose levels stimulate GHRH which stimulate GH pulsatility • Neural Inputs - Stress, exercise, deep sleep, malnutrition (combination of metabolic and neural) increase GH pulsatility
Other Hormones for Growth	**Optimal Growth** → requires normal concentrations of: • **Thyroid Hormones** - T3 and T4 - Needed at low concentrations for GH and IGF-1 to exert their normal growth effects - **Permissive role** in growth - Too much does not result in increased growth, but too little results in stunted growth • **Glucocorticoids** - Stress hormones such as cortisol - Low levels of cortisol required for normal growth

	- Excessive glucocorticoids result in reduced growth • **Sex Steroids** - Testosterone and estrogen - Act in concert with GH to stimulate growth at the tissue and hypothalamus levels • **Insulin** - Normal insulin levels required for normal growth - Deficiencies in insulin result in deficiencies in growth - Permissive role, promotes amino acid uptake into muscle (metabolic input for increased GH pulsatility)
Growth Abnormalities	**Growth Hormone Deficiency** → usually related to hyposecretion of GH, results in deficient growth • If GH deficiency occurs in adulthood, there are no major symptoms • If GH deficiency occurs in childhood, it will result in a phenotype of **pituitary dwarfism** - Short stature - Normal body proportions - Poor muscle development - Excess subcutaneous fat - If diagnosed pre-puberty, replacement therapy with recombinant hGH gives near normal growth
	Growth Hormone Excess → usually related to hypersecretion of GH from a pituitary tumour • GH hypersecretion in **children** results in: - Gigantism - Normal body proportions, because both long bones and soft tissues are affected • GH hypersecretion in **adults** results in acromegaly - Enlarged extremities: bones in hands, feet and face - Thickening soft tissues leads to coarse of malformed facial features
Prolactin	**Prolactin** → a pituitary hormone, a 199 AA polypeptide/small protein
	Prolactin Production → in the lactotroph cells of the anterior pituitary gland
	Prolactin Synthesis and Secretion → is inhibited by **dopamine** (prolactin inhibiting factor); is stimulated by unknown prolactin releasing factor • Recall these are neurohormones which are released from the hypothalamus into the median eminence