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Diabetes mellitus

Practicals – experimental diabetes mellitus in laboratory animal

Ústav patologické fyziologie LF MU

Definition of diabetes mellitus (DM)

- DM is a group of metabolic disorders characterized by **hyperglycemia** as a result of impaired effect of insulin
  - absolute
    - insulin is missing
  - relative
    - insulin resistance
    - impaired insulin secretion (gluco- and lipotoxicity)
- **chronic hyperglycemia** leads to cell & tissue damage (**complications**)
  - retina
  - kidney
  - nerves

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Diagnosis of DM

- classical symptoms of diabetes + random plasma glycemia ≥ 11.1 mmol/l
  - any time of the day
  - symptoms include polyuria, polydipsia and rapid loose of weight
- FPG (fasting plasma glucose) ≥ 7.0 mmol/l
  - fasting means at least 8 h from the last meal
- 2-h PG (postprandial glucose) ≥ 11.1 mmol/l during oGTT
  - according to WHO standard load of 75 g of glucose

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Interpretation of glycemia

- FPG:
  - < 6.1 mmol/l = normal glycemia
  - 6.1-7.0 mmol/l = **IFG (impaired fasting glucose)**
  - ≥ 7.0 mmol/l = diabetes

- oGTT – 2h PG:
  - < 7.8 mmol/l = normal glucose tolerance
  - 7.8-11.1 mmol/l = **IGT (impaired glucose tolerance)**
  - ≥ 11.1 mmol/l = diabetes

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Oral glucose tolerance test

- tool used for diagnosis of
  - diabetes mellitus
    - presence of diabetes in the family,
    - in obese patients and in hypertesion,
    - patients with glycemia 6.1 – 7.0 mmol/l twice in the row
  - gestational diabetes
    - early (< 12th week of pregnancy in women with at least 2 risk factors
      - age > 30 years
      - presence of diabetes in the family
      - macrosomia
      - obesity
      - diabetes mellitus in previous pregnancy
      - glycosuria
      - hypertension in previous pregnancy
      - repeated abortions
  - **“prediabetes”**
    - impaired glucose tolerance (IGT)
      - 2-h PPG ≥7.8 - <11.1 mmol/l during oGTT
    - impaired fasting glucose (IFG)
      - FPG ≥5.6 – <7 mmol/l
- procedure
  - FPG, drinking glucose solution (75 g + 250 ml water) within 5 – 10 min, glycemia measurement after 60 and 120 min

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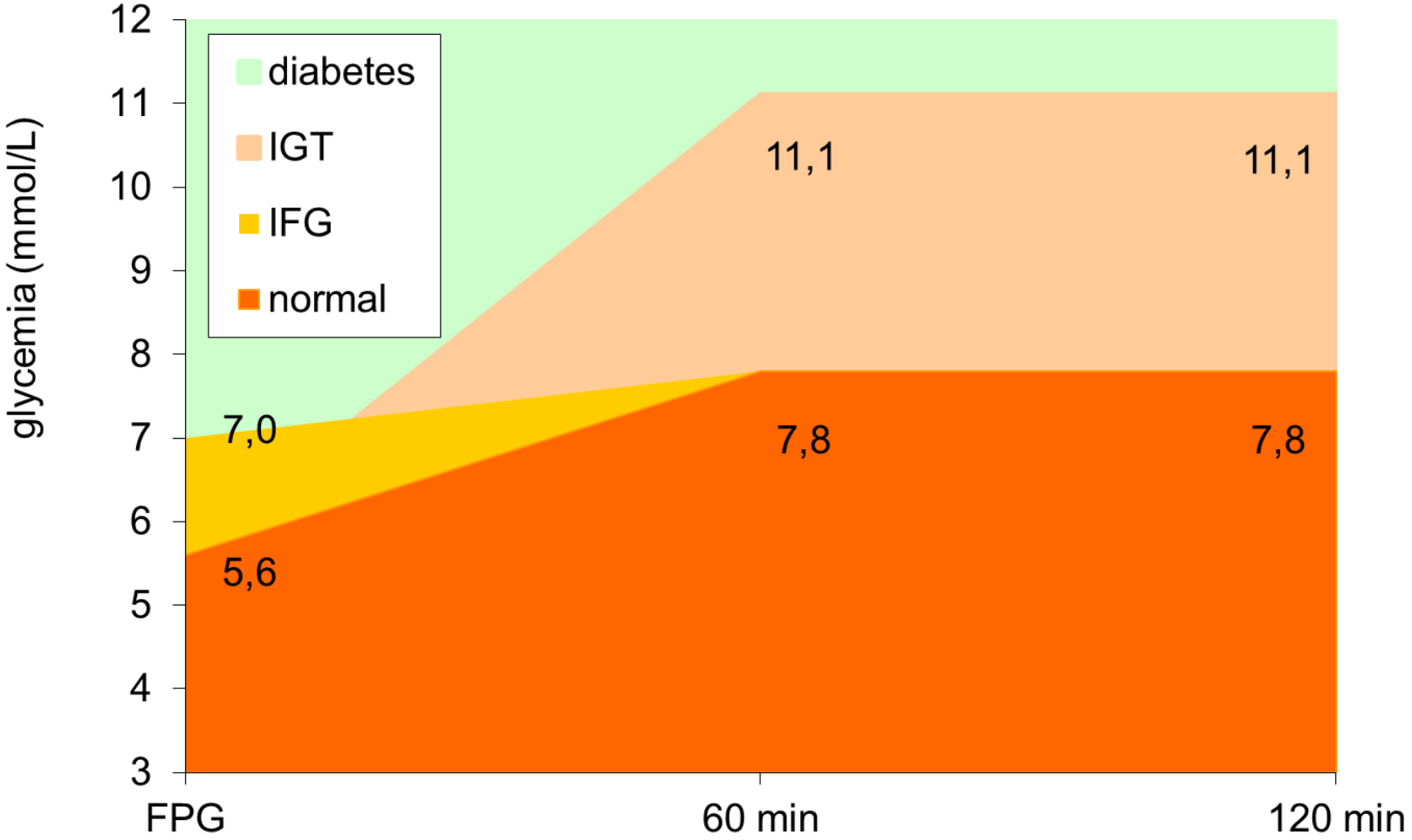
oGTT interpretation



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Animal models of type 1 diabetes

- 1889 – pancreas removal
  - Minkowski and Von Mering
    - diabetic syndrome in dog
- Banting and Best
  - insulin discovery and testing
  - the Nobel Prize in Physiology or Medicine 1923
- exp. diabetes induced in various species
  - β-cells toxins and viruses
- specific strains in which insulin-deficient diabetes develops spontaneously

Sir Frederick Banting and Dr. Charles Best
co-discoverers of Insulin

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Chemically induced insulin-deficient diabetes

- **alloxan**
  - first known diabetogenic chemical agent (1943)
    - islet-cells necrosis in rabbit
  - high doses
    - β-cells necrosis
  - acts on membrane and interior
    - inhibits insulin release
    - is taken up into the β-cell
      - glucokinase, PARP
      - free radicals
  - practical problems *in vivo*
    - instability at physiological pH
    - dosage variation with age and species
    - toxicity on other organs

- **streptozotocin (STZ)**
  - induces severe diabetes
    - i.v. or i.p.
  - most commonly used model
  - β-cell necrosis within 1-2 days
  - insulin falls to 10-30%
  - hyperglycemia 20-30 mmol/l
  - at dosage 50 mg/kg severe ketosis does not develop
    - survival without insulin replacement
  - may share cytotoxic mechanisms with alloxan

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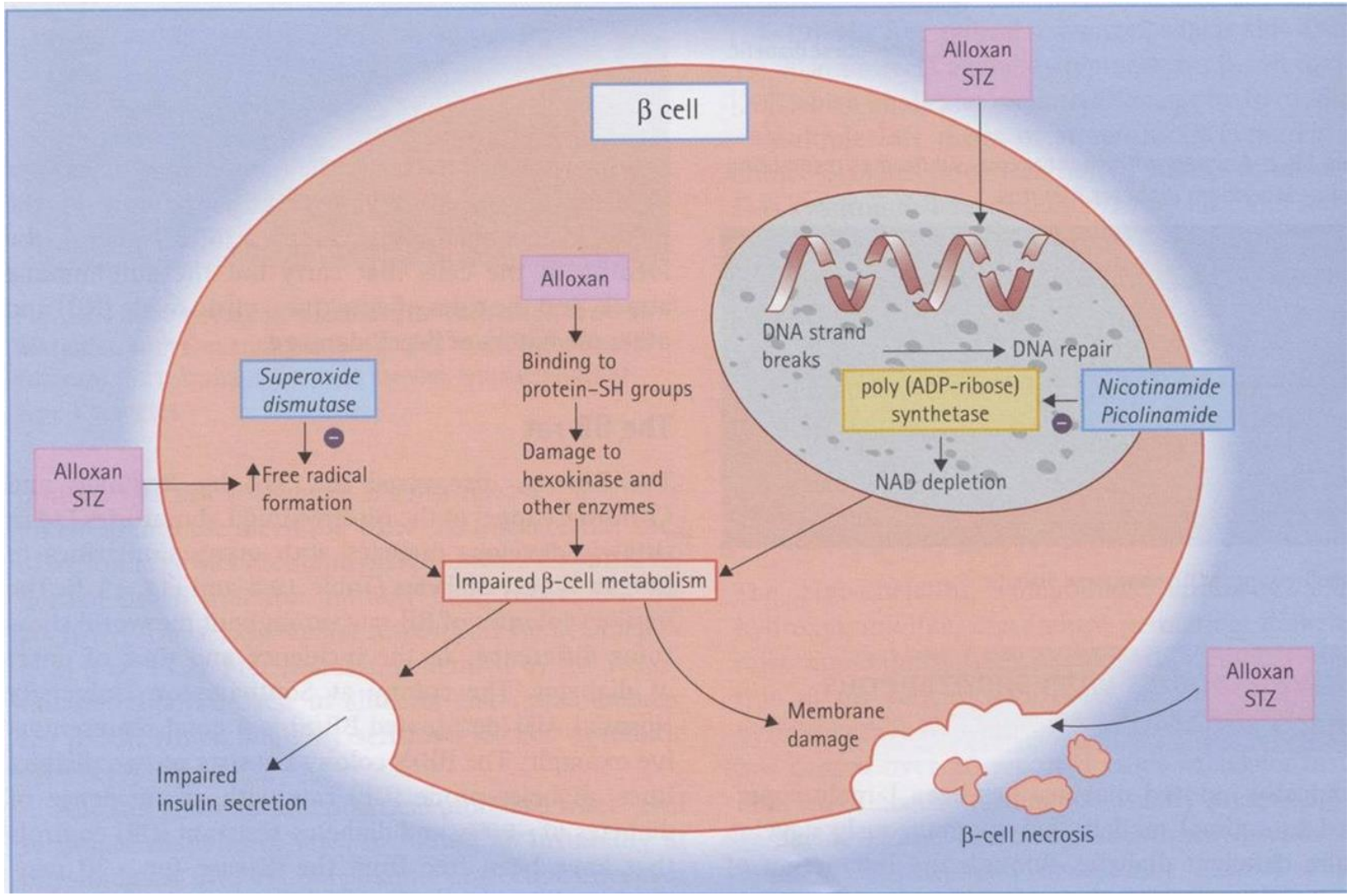
Mechanisms of alloxan and STZ action



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Animal models with spontaneous T1DM

- BB rat (BioBreeding)
  - defects in immunity
    - inflitration of islets with T lymphocytes
    - autoantibodies against GAD (glutamic acid decarboxylase) are present
    - severe hypoinsulinemia and hyperglycemia
  - two main T1DM susceptibility genes
    - 8 additional loci
- NOD mouse
  - non-obese diabetic
  - autoimmunie diabetes
  - diabetes develops as a result of insulitis
  - NOD mice will develop spontaneous diabetes when left in a sterile environment
  - affects 80 % of females and 20 % of males

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Summary of rodent models of T1DM

Induction mechanism	Model	Main features	Possible uses
Chemical Induction	High dose streptozotocin	Simple model of hyperglycaemia.	New formulations of insulin
	Alloxan		Transplantation models.
	Multiple low dose streptozotocin	Model of induced insulitis.	Treatments that may prevent beta cell death
Spontaneous autoimmune	NOD mice	Beta cell destruction due to an autoimmune process	Understanding genetics of type 1 diabetes
	BB rats		Understanding mechanism of type 1 diabetes
	LEW.1AR1/-iddm rats		Treatments that may prevent beta cell death
			Treatments that may manipulate autoimmune process
Genetically induced	AKITA mice	Beta cell destruction due to ER stress. Insulin dependent.	New formulations of insulin
			Transplantation models.
			Treatments to prevent ER stress
			(could also be used in type 2 diabetes research)
Virally-induced	Coxsackie B virus	Beta cell destruction induced by viral infection of beta cells	Establish potential role of viruses in the development of type 1 diabetes
	Encephalomyocarditis virus		
	Kilham rat virus		
	LCMV under insulin promoter		

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Summary of rodent models of T2DM

Induction mechanism	Model	Main features	Possible uses
Obese models (monogenic)	$Lep^{ob/ob}$ mice	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	$Lepr^{db/db}$ mice		Treatments to improve beta cell function
	ZDF Rats		
Obese models (polygenic)	KK mice	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	OLETF rat		Treatments to improve beta cell function
	NZO mice		Some models show diabetic complications
	TallyHo/Jng mice		
	NoncNZO10/LtJ mice		
Induced obesity	High fat feeding (mice or rats)	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	Desert gerbil		Treatments to improve beta cell function
	Nile grass rat		Treatments to prevent diet-induced obesity
Non-obese models	GK rat	Hyperglycaemia induced by insufficient beta cell function/mass	Treatments to improve beta cell function
			Treatments to improve beta cell survival
Genetically induced models of beta cell dysfunction	hIAPP mice	Amyloid deposition in islets	Treatments to prevent amyloid deposition
			Treatments to improve beta cell survival
	AKITA mice	Beta cell destruction due to ER stress.	Treatments to prevent ER stress
			Treatments to improve beta cell survival

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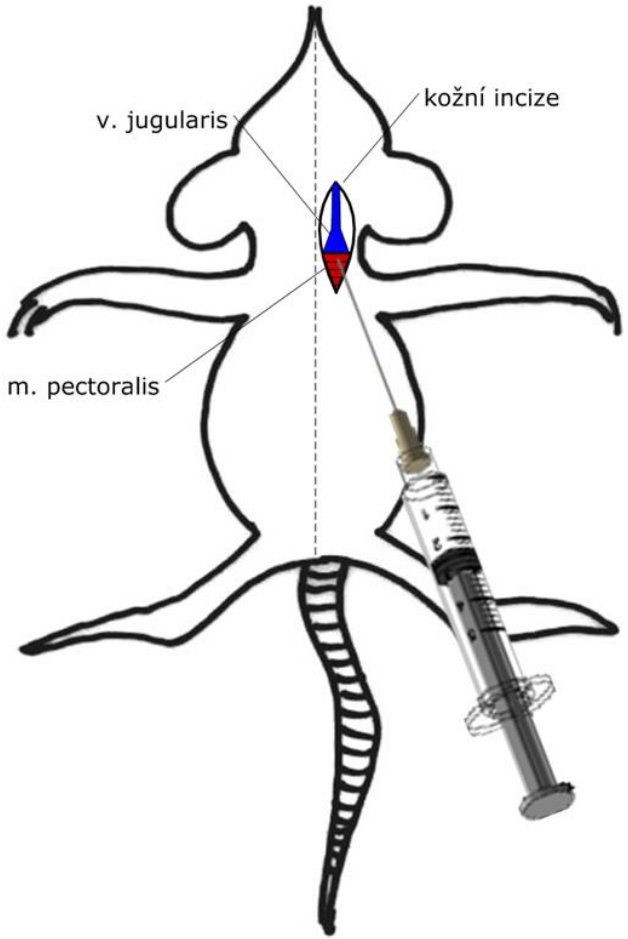
Experimental induction of DM



- anesthesia induction
  - Narkamon/Rometar, 0.5 ml/100 g of weight
  - intraperitoneal administration
- skin incision
- i.v. administration of **alloxan**
  - In the jugular vein
- suture
  - the wound is closed with several individual stitches
- second part of the experiment follows after 1 week

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The experiment