Molecules of Life and Mutations: a new course of advanced pathophysiology combining several modern didactic approaches

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INTRODUCTION

The study of medicine takes 6 yr in the Czech Republic (as well as in Austria and most other countries) and can be roughly divided into two halves, i.e., the preclinical and the clinical parts. Pathophysiology (together with pharmacology) can be regarded as the main subjects at the end of the preclinical phase. After passing all of the particular theoretical subjects (cell and molecular biology, genetics, biochemistry and biophysics, anatomy and histology, as well as basic physiology), students should by then be ready to integrate the entire knowledge gathered in the course of the first 3 yr of their study. Master students at the Faculty of Science do possess a compatible knowledge base from cell biology, histology, physiology, and some highly specialized subjects, such as immunology, genetics, bioinformatics, drug design, and general structural biology, which offers a possibility to design joint courses.

With this in mind, we conceived a new elective course of advanced pathophysiology (Fig. 1) and coined it “Molecules of Life and Mutations,” largely inspired by the textbook of Schwarz (8) that catalogs some 150 pathophysiology-relevant molecular structures. Our intention was to undertake several synthesis steps, allowing an integrated pathophysiological view on defined human diseases or other pathophysiological conditions.

The first synthetic focus was put on the structure-function relationships at the protein level. The ability to understand complex pathophysiological phenomena in terms of deviations of the underlying molecular structure is (not only for students) enormously helpful, and it strengthens the students’ notion of complex biological systems being, by and large, derivatives of numerous rather simple molecular interactions (1).

The second major point of synthesis was put on our aim to conceptually connect physiology and genetics. The ability to understand the behavior of mutated genes in pedigrees (mode of inheritance, completely and incompletely dominant or recessive, the issues of penetrance and expressivity, age of onset, genotype-phenotype correlation, etc.) could be essentially promoted by simultaneously understanding the physiological context in which the mutated protein(s) operates (9).

Thematical, we chose endocrinology as the main focus of the pilot run, essentially relying on the latest edition of the comprehensive endocrinology textbook (3), in part complemented with selected examples from original scientific literature. We have introduced the two synthetic principles mentioned above on three topics, each formulated into an introductory lecture featuring gradually increasing complexity. We started with the genetics and pathophysiology of diabetes insipidus, which allowed us to address and review the issues of posterior pituitary/hypothalamic hormone synthesis, secretion, and signaling, and the issues of kidney water reabsorption and homeostasis, making it possible for us to distinguish the central and the renal types of diabetes insipidus. What turned out to be especially revealing for the students was the question of genotype-phenotype correlation for clinically relevant mutations in the arginine vasopressin (AVP) gene, allowing us to explain in an extremely logical and straightforward way which mutation would result in a recessive inborn form and which one in a dominant, slowly progressing form.

The second lecture was devoted to growth hormone (GH) pathophysiology, which, by necessity, implied an increased complexity. First, GH is a good example to illustrate a complex endocrine circuitry in the form of hypothalamus-pituitary-peripheral organ axis, and, second, GH-GHR (growth hormone receptor), as well as IGF1 (or IGF2)-IGF1R receptor interactions and signaling are structurally more complex compared with AVP. In a direct extension to this, we could convince the students that understanding the regulation of GH synthesis as well as the action of GH in terms of structure-function relationships could have a dramatic impact on the design of drugs aimed to treat GH overexpression syndromes. Third, the Laron-type GH deficiency syndrome represents, from a pathophysiological point of view, a very valuable genetic disease to illustrate the plethora of physiological targets of GH beyond body growth, especially with respect to the mitogenic and cancer-promoting action of both GH and somatomedins (IGF1, IGF2), as well as with regards to diabetes mellitus or regulation of the immune functions. Last but not least, the IGF2–H19 locus provides an excellent example of genomic imprinting, allowing us to introduce the issue of epigenetic regulation, as well as addressing the loss of imprinting at the IGF2–H19 locus seen in certain tumors, to introduce to the students the concept of paraneoplastic syndromes exemplified by tumor-induced hypoglycemia.
The third and the most demanding lecture has been devoted to calcium and calcium homeostasis. This topic especially allowed us to build up molecular connections and concepts, which are usually only mentioned in a basic physiology course.

First, we could easily connect cell and organismal physiology by contrasting Ca\textsuperscript{2+} as a second messenger from its role as a direct signal at the calcium-sensing receptor (CaSR). Second, moving from the parathyroid hormone (PTH) and calcitonin to vitamin D synthesis and action, we could easily discriminate between different levels of feedback regulation, from direct molecular feedback (e.g., in form of direct transcriptional repression of the \textit{CYP24A1} gene and direct transcriptional repression of the \textit{PTH} gene), as well as the simultaneous direct activation of the \textit{CASR} gene, or by the vitamin D/FGF23 regulatory circuit.

In the next step, we addressed the question of Ca\textsuperscript{2+} homeostasis during lactation, which allowed us to introduce the PTHrP (PTH-related protein). Moreover, showing in detail the changes in CaSR-G-protein coupling in breast cancer cells (5), we were able to move to humoral hypercalcemia of malignancy as one of the most widespread paraneoplastic syndromes, and we mentioned also briefly the tumor-induced osteomalacia due to ectopic FGF23 expression. Moving to the genetics, we could use the two rare \textit{PTHR1}-mutation syndromes, Jansen and Blomstrand chondrodystrophy, to arrive to the remarkable positional feedback regulation at the bone growth plate between the PTH-related protein and Indian Hedgehog. Finally, we mentioned the famous example of calcitonin/calcitonin gene-related peptide to illustrate the power of alternative splicing in generating, from one gene, multiple proteins with totally disparate biological functions.

At the very end of the lecture part, we alerted the students to the multitude of reasons that could result in a particular endocrine disorder, distinguishing genetic and nongenetic (in a narrow sense) influence, and we illustrated it on two endocrinopathies discussed just before. Central diabetes insipidus could thus be encountered not only as a direct phenotypic expression of AVP mutations, but also as a consequence of pituitary trauma due to a surgical intervention, as well as a rare pregnancy complication, either due to placenta-produced vasopressinase, or arginine vasopressin-blocking antibodies. Likewise, familial hypocalciuric hypercalcelemia, a rare genetic disorder due to loss-of-function mutations in the \textit{CASR} gene, has its phenocopy counterpart in acquired hypocalciuric hypercalcelemia, an autoimmune endocrinopathy due to blocking anti-CaSR auto-antibodies. Autoimmune endocrinopathies thus allowed us to connect two crucial humoral systems ensuring organismal integrity: the endocrine regulation and the immune system.

The second part of the course served as a bridge between the introductory lectures by teachers and the student-centered final part of the course; it was previously shown that such a combinatorial teaching strategy has a very beneficial impact on the learning process (7). In this middle part, we introduced molecular modeling algorithms, especially RasMol (http://www.rasmol.org/software/RasMol_2.7.4/), and we resorted again to the AVP and diabetes insipidus example to illustrate the potential of molecular visualization (1). The goal of this part is to give rather detailed information in the form of a hands-on course in a computer room in which students learn how to use a particular molecular modeling software (RasMol/RasWin or several online tools, e.g., LiteMol) to display three-dimensional structures of proteins deposited in the Protein Data Bank, either alone or in interaction with small or large ligands, such as drugs or DNA, or other proteins. In particular, students are instructed how to display molecules, in total or in portions thereof, in different formats, such as wireframe, protein backbone, atoms, overall surface, etc. With such software, it is possible to turn the molecule in all directions and to see in real-time the various aspects of it, i.e., its outer surface as well as its inner pockets. Thereby, various structural characteristics can be recognized, such as domains of a certain structure, charge or hydrophobicity, shape, or other properties, which can serve as ligand-binding domain, DNA-binding domain, drug-metabolizing domain, or as a domain for any other biological purpose. Also, known mutations, as documented in the OMIM (Online Mendelian Inheritance in Man) and other databases, can be projected into such a model to understand which function of the protein would thus be altered and whether this change in structure would result in loss-of-function, gain-of-function, or dominant-negative effects, and finally lead to recessive or dominant inheritance patterns.

In the third, final part of the course, we adopted a problem-based learning strategy (2, 10) by asking students to elaborate a single molecule relevant for endocrine pathology from all of the points of view to which they have been introduced: pathophysiology, genetics, and structural biology. As a sort of guide, we put interesting candidates into a table (Table 1) containing hyperlinks to NCBI, HGNC Protein Data Bank, OMIM, and PubMed. On one hand, this was an immediate help to the students during the preparation of their cases; on the other hand, it allowed them to become familiar with these online resources, which have been only very briefly, if at all, mentioned in the first half of standard medical study. The students could freely choose their cases and were allowed to elaborate on these in a 3-wk home study period. This part was essentially unassisted by any tutor; nevertheless, both interactions and mutual help among students was encouraged, as well as between students and the course tutors, which was permitted, whenever students felt to be in need. At the end of this period, each student had to present his/her own case, either by being physically present in the classroom, or by means of an internet-based videoconference (4). Each student was encouraged to comment on other students’ case presentation, and it was the
Table 1. Representative overview of input information provided to students to work out chosen and assigned “molecular cases” out of 27 total

| Molecule (Link to NCBI)* | Gene Name (Link to HGNC)† | PDB ID‡ | Structure Description | OMIM ID§ | Disease-Related Allelic Variants |
|--------------------------|---------------------------|---------|-----------------------|----------|-------------------------------|
| Arginine vasopressin     | AVP (ID: 894)             | 1NPO    | Oxytocin + neurophysin 2 complex | 192340:  Arginine vasopressin | 125700: Diabetes insipidus centralis |
| Parathyroid hormone      | PTH (ID: 9606)            | 1HPH    | Human parathyroid hormone 1–37 in solution | 168450: Parathyroid hormone | Hypoparathyroidism, familial isolated |
| Platelet-derived growth factor beta polypeptide | PDGFB (ID: 8800) | 1PDG | Human platelet-derived growth factor B | 190040: Platelet-derived growth factor, beta | Meningioma, dermatofibrosarcoma protuberans |
| Growth hormone receptor  | GHR (ID: 4263)            | 3HHR    | Extracellular domain involved in hormone binding, dimerized | 600148: Growth hormone receptor | 262500: Laron syndrome |
| Thyroid stimulating hormone receptor | TSHR (ID: 12373) | 2XWT    | TSH receptor-antibody complex | 603372: TSH receptor | 275000: Graves disease susceptibility–hyperthyroidism |
| GNAS complex locus       | GNAS (ID: 4392)           | 1AZT    | Gs alpha + GTP gamma S | 139320: GNAS complex locus | Pseudohypoparathyroidism, pituitary tumor, 174800: McCune-Albright syndrome |
| Bruton agammaglobulinemia tyrosine kinase | BTK (ID: 1133) | 1BTK    | PH domain and Btk motif from Bruton’s tyrosine kinase | 300300: Bruton agammaglobulinemia tyrosine kinase | Agammaglobulinemia, X-linked |
| Androgen receptor        | AR (ID: 644)              | 1XJ7    | Ligand binding domain of human androgen receptor complexed to DHT | 313700: Androgen receptor | 1) Prostate cancer; 2) Androgen insensitivity syndrome; 3) Kennedy disease |
| Thyroid hormone receptor, beta | THRB (ID: 11799) | 1BSX    | Nuclear receptor T3R-coactivator interactions | 190160: Thyroid hormone receptor, beta | Thyroid hormone resistance, generalized |

HGNC, HUGO Gene Nomenclature Committee; ID, identification; NCBI, National Center for Biotechnology Information; OMIM,Online Mendelian Inheritance in Man; PDB, Protein Data Bank. *See https://www.ncbi.nlm.nih.gov/gene/. †See https://www.genenames.org/. ‡See https://www.rcsb.org/. §See https://www.ncbi.nlm.nih.gov/omim/.

Task of the course tutors to instigate and guide the debate (6). Illustrative student’s presentations are available on request.

The course was supervised by three tutors from three different faculties (Faculty of Medicine in Pilsen and Faculty of Science in Prague, both Charles University, Czech Republic, and Medical University of Innsbruck, Austria), and in the first pilot run, 16 medical students passed it. We believe that the course has the potential to mediate the transmission of skills and knowledge that will be extremely useful in the clinical part of their study. First, it allowed integration of partial knowledge gathered separately in different preclinical subjects. Second, it introduced students to the most accessible and universally useful general online resources. Third, it introduced students to the concept of problem-based learning. Fourth, by being forced to present their cases and respond to the questions from the audience (many of them being confronted with such a challenge for the first time during their study), students gained self-confidence, a necessary condition for their future clinical

Table 2. Student’s evaluation questionnaire and results

| Question                                                                 | 1 Fully Agree | 2 Agree | 3 Partly Agree | 4 Undecided | 5 I Do Not Agree |
|--------------------------------------------------------------------------|--------------|---------|----------------|-------------|-----------------|
| 1. During/after the course I have understood aspects of medicine that were, up until now, unclear to me. | 50.0         | 37.5    | 12.5           |             |                 |
| 2. I regard the three teachers as fully competent and devoted to the subject that they taught me. | 37.5         | 62.5    |                |             |                 |
| 3. I regard the style and type of the course as novel and important for my medical/biological understanding. | 68.7         | 31.3    |                |             |                 |
| 4. I would fully recommend other colleagues to attend this course.        | 37.5         | 37.5    | 25.0           |             |                 |
| 5. I regard the subjects chosen in this course as optimal and important examples to deepen my understanding in medicine/biology. | 18.7         | 50.0    | 31.2           |             |                 |
| 6. I would suggest that more and greater parts of the study of medicine/biology would follow such a style. | 81.2         | 18.8    |                |             |                 |

Values are in percent. Students’ evaluation on “Molecules Of Life” in Pilsen, 2018, is shown. Concerning an “explanation” for the slightly weaker responses to questions 4 and 5, it may well be that, within 3 days of this course, the topics chosen were, for some students, too difficult or too “far-fetched” or of too little “relevance” or not sufficiently covered in previous lectures in their home universities. It may also be that we have tried to put too much “material” into the limited time frame or that we have not been able to “convince” them sufficiently with providing relevant clinical examples or case descriptions. We will certainly take this result for a self-critical reevaluation and academic discussion among ourselves and with future students.

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decision-making ability, as well as collaboration between clinical practice and research. Finally, the course was held in English, and students from several European countries attended during their Erasmus+ stay, introducing especially the Czech students to the international community, which will be of great benefit to them onwards, a factor that might be relevant to all non-English-native universities.

At the very end of the course, we invited students to an open-feedback discussion. On this basis, and also based on our subjective impressions during the course, the course has generally been perceived very favorably. The major suggestions for improving this (annual) course in the future relate to trying to select a more homogeneous sample of student attendees, i.e., with regard to their study discipline (human medicine, biology, life sciences), year/progress of study, and perhaps language/country of origin. On the other hand, we feel that such differences, although regarded by some students as a certain shortcoming at their first look, could turn into an important asset, as they allow students to get at least a short glimpse into the other respective study directions, as well as into different countries of origin, which may be of importance for future interdisciplinary and/or international collaborations once the students become graduates and perhaps researchers. We also tried to perform a post festum formal evaluation using a short questionnaire. The response rate was, however, low, mainly, we assume, due to the long time lapse. Still, we think that the answers we received were very positive and reassuring (Table 2).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.H., K.D., and S.S. conceived and designed research; J.H., N.K., M.S.K., K.D., and S.S. performed experiments; J.H. and S.S. analyzed data; J.H. and S.S. interpreted results of experiments; J.H. and M.K. prepared figures; J.H., M.K., M.S.K., K.D., and S.S. edited and revised manuscript; J.H., M.K., N.K., M.S.K., K.D., and S.S. approved final version of manuscript.

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