2.1.

ANTINOICEPTIVE EFFECTS OF DALARGIN AND ANALOGUES

N. Pencheva, Topouzov, J. Barthova, T. Barth

(Submitted by Corresponding 1'vlember O. Poljakova on September 4, 2002)

Abstract

To elucidate the receptor preferences and structural features ensuring antinociceptive action of \([D-Ala^2, Leu^5]-Arg\) (dalargin), analogues of dalargin (dalarginamide and \([N-Me-Phe^4]\)-dalarginamide) were tested for their effectiveness in increasing the mechanical nociceptive threshold of rats measured by the Randall-Selitto paw-pressure test. All test compounds including endogenous \([Met^5]\)-enkephalin and mu-selective ligand DAMGO exerted well-pronounced elevation of paw-pressure threshold. The response profile of the opioids examined was similar; antinociception was maximal at the 10-min test interval except for DAMGO, which maximum effect was at the 15-min interval. Dalargin, dalarginamide and DAMGO were equipotent while \([N-Me-Phe^4]\)-dalarginamide was about 1.5 times more potent than them and 3 times than \([Met^5]\)-enkephalin. A low concentration of naloxone (0.5 mg/kg) antagonized the antinociceptive effects of all peptides, except that for \([Met^5]\)-enkephalin, suggesting that they are mediated by mu-opioid receptors.

Key words: opioids, enkephalins, nociception, paw-pressure test

2.2.

MODIFICATION OF THE PEPTIDE BOND IN N-TERMINAL PART OF \([8-D-\text{arginine}]\) DEAMINOVASOPRESSIN (dDAVP)

T. Barth, N. Pencheva***, J. Barthova***, J. Velek, J. Jezek, V. KaSicka, A. Machova, L. Hauzerova, K. Ubik

(Submitted by Academician E. Golovinsky on July 17, 2002)

Abstract

The synthetic analogue of \([8-L-\text{arginine}]\) vasopressin (AVP) \([8-D-\text{arginine}]\) deaminovasopressin (dDAVP)

\[
\text{Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-[D-Arg]-GlyNH,}
\]

has an altered profile of biological properties in comparison to AVP. It is used especially for the treatment of diabetes insipidus and for the activation of Factor VIII in haemophiliacs A. In contrast to intranasal administration, peroral application is complicated because dDAVP molecule is subjected to enzymic degradation in the gastrointestinal system. The molecule of dDAVP is protected against aminopeptidase, trypsin-like and endoprolylpeptidase degradation. The N-terminal part of the peptide chain appears to be vulnerable to cleavage by chymotrypsin-like enzymes that may disintegrate the peptide bonds. An analogue of dDAVP having a methylated nitrogen of the peptide bond between tyrosine and phenylalanine was synthesized on solid phase and characterized by physico-chemical methods and by antidiuretic assays [1]. The peptide had substantially lower antidiuretic activity than dDAVP. The
modification resulted in enhanced metabolic stability but decreased receptor recognition by several orders of magnitude.

Key words: synthetic analogues, [8-D-arginine] deaminovasopressin (dDAVP), [8-L-arginine] vasopressin (AVP), antidiuretic potency

В тази публикация са представени данни за ролята на модификацията в пептидната връзка в N-терминалната част на аналога на аргинин-вазоопресина – dDAVP (дезмопресин) върху неговата биологична активност, по отношение на метаболитната му стабилност, която има отношение към фармакокинетичните му характеристики.

2.7. AFFINITY AND AGONIST EFFICACY OF MU-SELECTIVE DALARGIN ANALOGUES

P. Milanov, N. Pencheva*, J. Barthova**, T. Barth***, A. Milanov****
(Submitted by Corresponding Member O. Poljakova on September 4, 2002)

Abstract. [D-Ala², Leu⁵]-enkephalyl-Arg (dalargin) and its analogues [D-Ala², Leu⁵]enkephalyl-Arg-NH₂ and [D-Ala², N-Me-Phe⁴, Leu⁵]-enkephalyl-Arg-NH₂ have been found to possess high potency and selectivity for mu-opioid receptors. The purpose of this study was to estimate the receptor affinity and relative agonist efficacy of these peptides in in vitro functional bioassay. The guinea-pig-isolated ileum-longitudinal muscle preparations were used. Cumulative dose-response relations obtained, were analysed by a procedure derived from a hyperbolic model of pharmacological agonism, which allows to calculate by explicit formulas agonist affinity (VKA) and relative efficacy (eA) of the peptides. Dose-response curves were approximated with two- and three-parametric hyperbolic functions. The results obtained showed: (i) two-parametric hyperbolic function with a constraint for more potent compounds allows a good approximation of the dose-response relations obtained and easy calculation of the parameters KA and eA; (ii) substitution of COOH terminal in the molecule of dalargin with NH₂ group increased about two times the potency and five times the efficacy of [D-Ala², Leu⁵]-enkephalyl-Arg-NH₂, as compared with dalargin; as light decrease of affinity is also observed; (iii) N-methylation of the aromatic group of Phe together with CONH₂ terminal extremely increase the potency (about 34 times), affinity (4.4 times) and efficacy (six times) of the analog [D-Ala², N-Me-Phe⁴, Leu⁵]enkephalyl-Arg-NH₂, as compared with dalargin. The results of these analyses permitted us to observe the effect of structural modification on both receptor affinity and relative agonist efficacy to mu-opioid receptors. Affinity and efficacy should be incorporated as a drug design considerations for the selective opioid agonists.

Key words: opioids, enkephalins, dalargin, affinity, efficacy, curve fitting

2.8. MOLECULAR DESIGN OF SELECTIVE ENKEPHALIN ANALOGUES BY NEURAL NETWORK MODELLING

P. Milanov***, N. Pencheva****, I. Trenchev*, A. Milanov****
(Submitted by Academician P. Kenderov on January 21, 2004)

Abstract. The theory of artificial neural networks as an interconnected assembly of simple processing units (whose functionality is loosely based on the animal neuron) is briefly reviewed focusing on
techniques which have great impact on current chemical applications. The objects of neural network modelling in this study are endogenous peptides and their analogues with morphine-like properties (enkephalins). Explicit formulas for calculation of the pharmacological agonism of these peptides were presented. Design of network type and architecture for modelling of sequence-activity, sequence-selectivity and sequence-efficacy relationships in enkephalin analogues is further developed by nonlinear modelling of quantitative structure-activity relationships using a multilayered feed forward network. The type of the so-called transfer or fitness functions are determined in the different layers of the network. Advantages of more complex network architecture which allow to predict potency, efficacy and selectivity of the enkephalins were characterized.

**Key words:** neural networks, molecular design, drug design, network architecture, transfer function

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### 2.17.

**ANTINOCICEPTIVE EFFECTS OF DES-OCTAPEPTIDE-INSULIN CONNECTED WITH ENKEPHALINS**

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**Abstract.** Three insulin analogues with modifications of the B-chain were synthesized by trypsin-catalyzed coupling of desoctapeptide (B23-B30)-insulin (DOI) with Met5-enkephalin (Met5-enk) or Leu5-enkephalin (Leu5-enk). The derivatives DOI-Met5-enk and DOI-Leu5-enk were prepared by a condensation between the amino group of the enkephalins and the carboxyl group of arginine in position B22. To test the properties of DOI as a navigating molecule of active opioid peptides, we examined DOI, DOI-Met5-enk and DOI-Leu5-enk following three types of *in vivo* nociceptive methods: writhing test in mice, paw-pressure test and hot plate test in rats. The peptides were administered both intracerebroventricularly (icv) and subcutaneously (sc). The ability of the peptides to inhibit the electrically-evoked contraction in guinea-pig ileum and mouse vas deferens was also tested. To prove the opioid nature of the responses we used naloxone (1 mg/kg intraperitoneally). Using paw-pressure and hot-plate test, all compounds exerted wellpronounced antinociceptive effects (DOI < DOI-Met5-enk < DOI-Leu5-enk), with duration at least 40 min after icv application. The differences in order of potency were established after sc application and the effects of all compounds were developed for 20 min. The results obtained with writhing test in mice showed that all compounds did not influence the visceral pain. *In vitro* effects were poor and were observed at concentration higher than 20 μM for DOI-Met5-enk and higher than 100 nM for DOI-Leu5-enk. The present results suggested that the derivatives of DOI: DOI-Met5-enk and DOI-Leu5-enk achieved prolonged antinociceptive action, while DOI at some extent could be used as a transport molecule across the blood-brain barrier.

**Keywords:** des-octapeptide insulin; DOI; Met5-enkephalin; Leu5-enkephalin; pain; antinociception.

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2.23.
THEORETICAL HYPERBOLIC MODEL OF A PARTIAL AGONISM: EXPLICIT FORMULAS FOR AFFINITY, EFFICACY AND AMPLIFICATION

Peter Milanov, Nevena Pencheva

Abstract. The quantitative analysis of receptor-mediated effect is based on experimental concentration-response data in which the independent variable, the concentration of a receptor ligand, is linked with a dependent variable, the biological response. The steps between the drug–receptor interaction and the subsequent biological effect are to some extent unknown. The shape of the fitting curve of the experimental data may give some in-sights into the nature of the concentration–receptor–response (C-R-R) mechanism. It can be evaluated by non-linear regression analysis of the experimental data points of the independent and dependent variables, which could be considered as a history of the interaction between the drug and receptors. However, this information is not enough to evaluate such important parameters of the mechanism as the dissociation constant (affinity) and efficacy. There are two ways to provide more detailed information about the C-R-R mechanism: (i) an experimental way for obtaining data with new or selective or inactive compounds; and (ii) a theoretical way by making addi-tional assumptions and experimental observations about some elements of the C-R-R mechanism. Using the second way and basic postulates of the so-called occupation theory, a Theoretical Hyperbolic Model (THM) was developed in this study, in order to justify the nature of partial agonism in in-vitro bioassay studies. The model could be used for sensitive analysis of the partial agonist’s behav-ior from the experimental dose-response data. The explicit formulas derived from the model describe the affinity and relative Stephenson’s efficacy. Moreover, THM allows estimation of the receptor reserve of the almost full agonists under the assumption presented. When the design of the in-vitro bioassay allows assessing the maximal possible effect of a given isolated tis-sue, the affinity and relative efficacy of the respective partial agonists could be calculated from the experimental dose-response data. It was proved theoretically that the partial agonists have no receptor reserve. This finding confirms experimental results for partial agonists with varying potency. The THM is used further to explain the C-R-R mechanism and to understand more deeply the character of the affinity and efficacy of the agonists by in-trduction of a new agonist feature called amplification and the parameter amplifier. The THM is compared with other models related with the occu- pation theory of agonism. The differences and limitations of the application of THM are discussed.

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2.24.
COMPUTER MODELING OF LIGAND-RECEPTOR INTERACTIONS – ENKEPHALIN ANALOGUES AND DELTA-OPIOID RECEPTOR

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ABSTRACT. Since, Hughes determined endogenous opioid pentapeptides – enkephalins, large number of synthetic analogues were prepared. Many analogues of enkephalins were synthesized by our group in addition. In our previous study we established a relationship
between the replacement in position 2 in endogenous enkephalins and their \( \delta \)-opioid receptor selectivity. Computer modeling was used in this study to analyze binding affinity of a series of \( \delta \)-opioid selective enkephalin analogues to the model of \( \delta \)-opioid receptor, published in PDB (id: 1OZC). MolDoc SE algorithm implicated in the software program Molegro Virtual Docker was used. Basing on docking results was established that: 1) all enkephalin analogues have good binding affinity to \( \delta \)-opioid receptor by forming H-bonds with specific amino acid residue in the receptor pocket; and 2) the rank of the derivatives obtained with this approach is rather different compared with the rank of their biological in vitro assay activity. These results reveal further steps for the computer modeling of selective encephalin analogues such as: 1) development of a novel optimization procedure; and 2) application of a different algorithm and software.

**Key words:** enkephalins, delta-opioid receptor, computer modeling, docking

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3.3.

**Design and synthesis of novel Arg-Gly-Asp containing analogues of the fragment (296-306) of the glycoprotein IIb membrane**

Mihail L. Mondeshki, Nevena Pencheva and Ljubomir T. Vezhenkov

RGD is a key chemical signal for recognition of a variety of receptors, located on the surface of platelet membrane. Natural and synthetic peptides containing this sequence are antagonists to the complex Gp IIb/IIIa and can inhibit aggregation to a different extent. In the present study, we synthesized a peptide containing the sequence of the fragment of 296-306 of the Gp IIb - Val-Phe-Arg-Gly-Asp-Gly-Arg, which includes the antiparallel structure of the RGD motif, analog of 296-306 fragment of the thrombopoietic glycoprotein Gp IIb - Val-Phe-Arg-Gly-Asp-Gly-Arg, which is efficient in inhibiting aggregation (IC50 = 45 ± 9 nM), and has a better effect in vitro compared to active factors of the fibrinogen degradation products (APTT 15 ± 4 seconds, PT 23 ± 6 seconds and TT 4 ± 1.5 seconds) and does not affect the level of fibrinogen in human plasma.

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3.5.

**Design, Synthesis and Biological Activity of Analogues of Tick Anticoagulant Peptide (1-9)**

Emilia NAYDENOVA, Lyubomir VEZENKOV and Nevena PENCHEVA

Tick anticoagulant peptide (TAP) is a specific Factor Xa inhibitor. The new analogs of TAP(1-9) combining both 1-9 residues of TAP and active fragment o-Phe-Pro-Arg were...
AMINODERIVATIVES OF CYCLOALKANESPIROHYDANTOINS: SYNTHESIS AND BIOLOGICAL ACTIVITY

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Abstract

3-Aminocycloalkanespiro-5-hydantoins were synthesized and their biological activity was studied. In contrast to hydantoins, these compounds failed to induce either anticonvulsive effects in the central nervous system or inhibitory effects on cholinergic contractions in the enteric nervous system. However, they exerted well pronounced, atropinsensitive, contractile effects on the guinea-pig ileum longitudinal muscle preparations. Structure-activity relationships established allow the assumption that: (i) the reduction of the ring size in the molecule of the spirohydantoins leads to an increase in the potency of the respective analogue to induce contractile effect; (ii) the introduction of NH₂ in position 3 increases the ability of all the compounds studied to exert contractions; (iii) the enlargement of the ring leads to: (I) an increase of the degree of desensitization of the preparations; and (2) a decrease (except Ia) of the potency of the analogues to exert contractile effects. © 2002 Editions scientifiques et medicales Elsevier SAS. All rights reserved.

Keywords: Anticonvulsive activity; Contractile effect; Hydrazine reaction; NH-group reactivity

3.7.
The Interaction of dDAVP Analogues with Human Platelet Vasopressin Receptors

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A series of dDAVP -((8-D-arginine)deaminovasopressin) analogues with modifications in Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-[D-Arg]-GlyNH₂ positions 2 and 8 were synthesized. Tyrosine in position 2 was replaced by L or D p-alkyl phenylalanine or by stereoisomers of tyrosine analogues and D-arginine was substituted by D-homoarginine. The substances had a remarkably different profile of biological properties as...
compared with dDAVP (decreased antiuretic potency, strong inhibitory effect in the uterotonic assay, etc.). Some of the peptides were also tested for their ability to activate Factor VIII and release the tissue platelet activator. We present results of the interaction of dDAVP analogues with the platelet vasopressin receptors. [8-L-Arginine]vasopressin (AVP) caused a concentration dependent aggregation in human heparinised platelet rich plasma, the IC₅₀ value for AVP was 30 ± 12 nM (Student's t-test). dDAVP decreased the AVP induced aggregation response and its IC₅₀ value was 660 ± 84 nM. [8-D-homoarginine] deaminovasopressin was a better inhibitor with a IC₅₀ value of 370 ± 56 nM. Both structural modifications contributed to stronger inhibition of AVP induced platelet aggregation. Modifications in position 2 of [8-D-Har]deaminovasopressin resulted in peptides which bound to the AVP platelet receptor even more strongly than AVP.

In this investigation was undertaken to characterize the arginine vasopressin (AVP)-induced response and the effects of theophylline in platelets anticoagulated with citrate or heparin. In heparinized platelet rich plasma (PRP), AVP induced a pronounced effect (max. aggregation = 88 ± 10%; ECₛₒ = 30 ± 4 nM), while in citrated material the response was poor (max. aggregation = 10-19%; ECₛₒ = 65 ± 18 nM and 95 ± 22 nM in female and male subjects, respectively). Theophylline 0-100 μM did not change or decrease the response of AVP in heparinized PRP, but in citrated material potentiated this response by 50% in female subjects. Selective V₂ receptor analogue [1-deamino, 8-D-arginine] vasopressin (dDAVP, 1000 nM) shifted to the right the dose-response curve of AVP (ECₛₒ = 30±12 nM and 420 ± 83 nM, before and after dDAVP, respectively). Thus, the platelets could be a useful model for testing vasopressin receptors and allow us to further explore the role of vasopressin in homeostasis. Keywords: Vasopressin; Platelet aggregation; Heparin; Theophylline; Vasopressin receptors.

3.8.
VARIABILITY OF THE HUMAN AGGREGATION RESPONSE OF NORMAL PLATELETS TO AVP
Nevena PENCHEVA a,b, Tomislav BARTH c, Jana BARTHovA c,d, Marin PENEV e and Antoaneta MLADENOV e

MLADENOV e

This investigation was undertaken to characterize the arginine vasopressin (AVP)-induced response and the effects of theophylline in platelets anticoagulated with citrate or heparin. In heparinized platelet rich plasma (PRP), AVP induced a pronounced effect (max. aggregation = 88 ± 10%; ECₛₒ = 30 ± 4 nM), while in citrated material the response was poor (max. aggregation = 10-19%; ECₛₒ = 65 ± 18 nM and 95 ± 22 nM in female and male subjects, respectively). Theophylline 0-100 μM did not change or decrease the response of AVP in heparinized PRP, but in citrated material potentiated this response by 50% in female subjects. Selective V₂ receptor analogue [1-deamino, 8-D-arginine] vasopressin (dDAVP, 1000 nM) shifted to the right the dose-response curve of AVP (ECₛₒ = 30±12 nM and 420 ± 83 nM, before and after dDAVP, respectively). Thus, the platelets could be a useful model for testing vasopressin receptors and allow us to further explore the role of vasopressin in homeostasis. Keywords: Vasopressin; Platelet aggregation; Heparin; Theophylline; Vasopressin receptors.
ендогенната опиатна система и участнието й във физиологични и патофизиологични реакции, които съпътстват физическото натоварване, физиотерапевтичните процедури, както и изобщо посъществуващата активност и локомоцията.Освободените опиоиди участват в регуляцията на т. нар. „post-exercise“ хипотензия, усещането за болка, интинмирото механизми и метаболитните процеси на мускулното съкрашение, настроението и психическата статус, естествения имунитет и др. Тези модулиращи въздействия на ендогенните опиати са регуляторен, автотерапевтичен отговор, който е важна част от общия адаптационен синдром в организма на животните и човека.

Ключови думи: ендогена опиатна система, опиоиди, опиоидни рецептори, енкефалини, ендормфини.

2.9.

ОПИОИДНИ МЕХАНИЗМИ НА АДАПТАЦИОННИТЕ ПРОМЕНИ ПРИ ФИЗИЧЕСКО НАТОВАРВАНЕ

Невена Пенчева и Любка Божкова

В тази обзорна статия са представени адаптационните промени, които толерират стрес-индюционите реакции при физическо усилие, физическо натоварване и спортна тренировка, като противодействие на разстройствата в хомеостазата. Представени са експериментални данни върху: - ендогенната опиатна система (пептиди и рецептори); - промени, които се медират от ендогенни опиоиди при аеробни и анаеробни натоварвания; - физиологични механизми на адаптация, които се медират от опиоиди и предизвикват аналгезия и еуфория, промени в активността на буферните системи, метаболитни регулации, които предизвикват оптималното утилизиране на глюкозата и мастните киселини, модулации върху симпатоадреномедуларната система, поддържането на скелетно-мускулната система и др.

Трансформацияте в метаболизма, които се резултат на един автофармакологичен отговор медиран от ендогенните опиоиди са представени в обобщено схема, където повишаването на работния капацитет при физическо усилие е резултат от повишен толеранс към физическото натоварване чрез ефекти върху скелетно-мускулното съкрашение, вентилаторния праг, нюциптивния праг, алкално-киселинния баланс и ацидозната поносимост и др. Тези данни определят модулиращите влияния на ендогенните опиоиди при физическо усилие и спортна тренировка като съществени за адаптационните промени водещи до повишаване на издържливостта и толеранса към интензивни и продължителни усилия и потенциране на ергометричната работоспособност.

2.10.

Опиоидни медиаторни механизми на аналгезия и еуфория при физическо натоварване

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РЕЗЮМЕ
Медиацията на стимули въздействащи върху ендогенните системи за болка и удоволствие се опосредства на спинално и супраспинално ниво от ендогенната опиоидна система. При физиологични състояния тя се активира в най-голяма степен от физическо натоварване, проприорецептивни стимулатии, редица кинезитерапевтични и физиотерапевтични подходи, храна и секс, които провокират освобождаването на ендогенните опиоиди. Те имат своята роля, както за модулацията на болковата перцепция, респективно за лечение на болката, така и за генериране на чувство на еуфория. Настоящата обзорна статия има за цел да представи накратко съвременните схващания по тази проблематика като разгледа следните нейни страни: (а) невротрансмитерни и невромодулаторни механизми на болката; (б) опиум, опиати, опиоиди и опиоидни рецептори; (в) участие на опиоидите в сензорните пътища, повлияващи болката; (г) опиоиди и еуфория, психическа и физическа зависимост, ноцицепции; (д) физическо натоварване, хомеостаза, ноцицептивни стимули, опиоиди; (е) опиоидна модулация на физическото усилие при натоварване. Проследени са също промените в хомеостазата при физическо натоварване, които играят роля на ноцицептивни стимули и повишават освобождаването на опиоиди. Това повишава толеранса към физическото усилие, подобрява анаеробния капацитет и повишава издържливостта и ергометричната работоспособност.

Задълбочени изследвания върху опиоидния отговор при физическо натоварване: (а) биха прецизирали критерияте за работоспособност в областта на спорта и трудовата хигиена; (б) биха разкрили интимни биохимични механизми на кинезитерапевтичните и физиотерапевтичните подходи при лечение на социално-значими заболявания на опорно-двигателния апарат, сърдечносъдовата и дихателната система и др. и (в) са предпоставка за повлияване на стрес-индуцирани заболявания и наркомани.

Ключови думи: физическо натоварване; хомеостаза; опиоиди; ноцицепция; еуфория.

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2.11.

ДИНАМИКА В ПЕРЦЕПЦИЯТА ЗА БОЛКА ПРИ НАТОВАРВАНЕ
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Целите на настоящото изследване са: - да оцени влиянието на интензивно физическо натоварване върху болковия праг (PT) и болковия рейтинг; и – да проследи сърдечната честота (HR), систоличното и диастоличното кръвно налягане (BPs, Bpd), максималния ергометричен капацитет (Wmax) и нивата на лактат преди и след натоварването. Дванадесет мъже, спринтьори, със средно ниво на тренираност и дванадесет нетренирани мъже на възраст 20-24 години, височина 177±2.8 см и телесна маса 75±4.8 kg, участваха в изследването. Тренираните (TR) и нетренираните (UTR) участници бяха тествани преди и след максимален,
стъпала на ниво от 60%\textsuperscript{a} и съдържащ степените много силна болка и силна болка, умерена по сила болка и отсъствие на болка. Беше определен кумулативно след напомпване на маншет от апарат на кръвно налягане и сърдечна честота. Стойностите, получени за максималната концентрация в кръвта след напомпване, съответстваха на вербалната оценка на изследваното лице, а болковият рейтинг, оценен с VRS беше измерен след 3-минутна исхемия на ниво наRR\textsubscript{a}+50 mm Hg. Данните за различията между средните стойности в групите бяха анализирани с дисперсионен анализ (ANOVA) и Student t-test (p<0.05). Бяха получени следните резултати: (1) PT след натоварване (пет минути) беше значително по-висок (p<0.05) в сравнение с този преди натоварване, както при тренирани, така и при нетренирани; повишението се отнасяше за всички измерени нива и беше както следва [%]: UTR - APT-19.7±2.0; PPT - 23.5±2.4, TRPT - 15.9±1.8 и PPTPT - 15.9±1.8 и PPTPT - 15.5±1.9; TR - APT-TRPT-14.3±2.1, PPTTRPT - 14.3±2.0, TRPT - 30.0±1.8 and PIPT - >30, но не беше определен (p>0.05); (2) болковият рейтинг след VRS беше значително по-низък 10 минути след натоварването при двете изследвани групи; (3) повишението в болковия праг след натоварването при TR беше по-манифестирано (p<0.05) на отношение на PTPT и PPTPT, т.е. при по-мощните болкови стимули, докато при UTR беше по-изразено за APT and PPT; (4) стойностите, получени за PTPT and PPTPT преди натоварването бяха по-високи при TR в сравнение с тези при UTR лица. 

Тези данни са в съответствие със: стойностите получени за Wmax (300±12 W for TR and 190±14 W for UTR); - динамиката в сърдечната честота (проследена чрез EKG регистрация; стойностите получени за максималната HR бяха 187 ±2 уд/min при UTR и 186±2 уд/min при TR); и – с нивата на лактатните концентрации в кръвта след натоварването (La = 7.9±2.5 mmol/l при TR и 5.4±1.4 mmol/l при UTR).
2.14. **EXCENTRICAL MUSCLE CONTRACTIONS: PHENOMENA AND THEIR ROLE IN SPORT AND REHABILITATION**

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**Rézumé.** Skelletnите мускули генерират сила чрез промени в пространствената конфигурация на съкратителни белтъци. Еластичните компоненти на цитоскелета на мускулните влакна предават съкращенията на мускулната тонус и пр., т. е. мускулния тонус. Когато големината на сила, приложена към скелетен мускул е по-голяма от тази, която той може да генерира, мускулът ще се удължи и в този случай казваме, че извършва удължаващи или ексцентрични съкращения. В настоящия кратък обзор са представени съвременни данни за свойствата на ексцентричната мускулна работа, която има феноменален характер защото: (a) генерира най-голяма сила и мощност в мускулите, в сравнение с другите типове съкращения; (b) има най-ниски метаболитни разходи; и (c) кислородната консумация, която е лимитиращ фактор при физически натоварвания, при нея е най-малка. Макар, че физиологичните, биомеханичните и биохимическите аспекти на ексцентричните съкращения са спорни, по-нататък в обзорната статия са разгледани участието и приложенията на ексцентричните натоварвания в: (a) плиометричната тренировка, използвана при различни спорти дисциплини; (b) kinesiterapiята при хипертрофия, свързана с прогресивна загуба на мускулна маса при възрастни, при остеопения и мускулно-сухожилини увреди и пр.; и (c) реабилитацията на пациенти с кардио-респираторни дефекти, поради щадящия режим на работа на сърдечносъдовата и дихателната система. Фундаменталните страни на ексцентричните мускулни съкращения и участието им в спорта и реабилитацията са актуални теми, но слабо застъпени в литературата на български език, което прави настоящата обзорна статия полезна.

**Ключови думи:** ексцентрични съкращения; плиометрия; стречинг; хипертрофия, саркопения.

2.15. **Experimental pain measurement in different exercise models**

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Abstract
The cuff pressure algometry for the assessment of experimental pain, before and after exercise, has been studied using the following parameters: discomfort (DSC), pain thresholds (PT), powerful tolerable pain (PTP), powerful intolerable pain (PIP) and pain rating (PR), in both - eccentric loading of the elbow flexors and graded dynamic exercise to exhaustion. DSC, PT, PTP and PIP were significantly higher and PR was significantly lower (p<0.05) after graded exercise. The hypoalgesic effects were pronounced for trained and for more powerful pain stimuli. Eccentric loading evokes hyperalgesia and the dynamics in the pain parameters was most manifested 24 hours after exercise. So, the tourniquet algometry is a sensitive and noninvasive method for measurement of experimental pain in different models of exercise.

Keywords: Cuff pressure algometry; pain threshold; pain tolerance; eccentric exercise; incremental exercise

BLOOD GASES AND ACID-BASE STATE BEFORE- AND AFTER GRADED DYNAMIC EXERCISE

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Abstract
The aim: to examine the effect of an ergometric test on exhaustion in untrained (UTR) and trained (TR) males on blood gases, acid-base state (pH; HCO3−; BEb) and blood level of lactate - Lab. The activity of creatine kinase (CK), lactate dehydrogenase and their isoenzymes were also determined. Runners and UTR males performed incremental test. Arterialized capillary and venous blood samples were drawn and assessed. Lab after exercise were higher in TR and corresponds to longer duration and higher maximal exercise power (270.0±28.3 W, compared with 216.0±12.6 W in UTR). The decrease in pH was bigger in UTR. The increased CK in UTR, probably originating from the skeletal muscles, was established. The [BE]/[La]b ratio indicates deadaptation state for UTR and very good adaptation for TR.

Keywords: Blood gases; acid-base state; lactate concentration; base excess; creatine kinase.

STIMULUS-RESPONSE RELATIONS OF DEEP TISSUE PAIN OBTAINED WITH COMPUTER-CONTROLLED PRESSURE ALGOMETRY

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Abstract: The aim of this study was to test the newly designed computerized pressure algometer. The study included healthy male subjects. The experimental set-up consisted of a tourniquet cuff, a computer controlled air compressor and an electronic visual analogue scale. In case of 1 kPa/s and with biofeedback, the values for the pain threshold (PT) and pain threshold tolerance (PTT) were: 206 ± 10 kPa and 243.7 ± 13 kPa respectively. An increase (p<0.05) of PT was established following the changes of compression rates. The values without biofeedback were similar concerning PTT, while the values for PT were without changes. The optimal approximation of the pressure-pain data was obtained with exponential growth function (y=y0+Aexp(x/t)). The presented study allow: (i) to obtain and analyze experimental stimulus-response data with computerized cuff pressure algometer; (ii) to established that in healthy volunteers the pressure-pain data could by approximated with exponential growth function; (iii) to precise the range of PT and PTT without and with biofeedback.

Keywords: pressure algometry, deep tissue pain.

2.19.

Microcontroller-based system for pneumatic pressure algometry

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Abstract

Although the human pain research on deep tissue pain by pressure stimulation is relevant in experimental and clinical settings, there are no data in the literature, describing the theoretical basis, experimental set-up and testing of contemporary computerized system for pneumatic pressure algometry. That is why in this article a microcontroller-based, cuff pressure algometer is proposed. Details and solutions for the components of the microcontroller-based data acquisition system, design of the microcontroller and electropneumatic regulator, considerations and solutions for the software and for the development environment and requirements for the hardware, are presented. The experimental set-up consists of: pneumatic tourniquet cuff, a computer-controlled air compressor and a 100 mm electronic visual analogue scale (VAS) for continuous pain rating. The range of the compression rate is 0-5 kPa/s. Further, we tested the device by a preliminary study with healthy, male volunteers and obtained values for pain threshold and pain tolerance threshold, assessed on the astrocnemius-soleus muscle of the right leg, as a function of compression rate (0.25, 0.50 and 1.00 kPa/s). The pressure-time VAS curves were also obtained. These results give us ground to believe that the microcontroller-based, pneumatic algometer, proposed in this article is a contemporary device for cuff pressure algometry and for quantitative pain testing with possibilities for assessment of temporal and spatial sides of deep somatic or muscle pain by stimulus-pain response curves.

Keywords: microcontroller, data equisition system, development environment, cuff pressure pneumatic algometer, pain threshold, pain tolerance threshold, pressure-time VAS curve.
ОЦЕНКА НА ДЪЛБОКА ТЪКАННА БОЛКА ПРЕДИ И СЛЕД НАТОВАРВАНЕ ПРИ ЗДРАВИ ХОРА

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Резюме. Представени са данни за оценка на експериментална дълбока тъканна болка при механична стимуляция на здрави нетренирани (n=12) в покой и след субмаксимално, стъпаловидно, натоварване измерени с компютъризиран, пресорен алгометър. Устройството се състои от пневматичен регулатор, турникетов маншет, компрессор и електронна, визуално-аналогова скала. Маншетът се намотава около левия и десния крак на нивото на m.gastrocnemius-m.soleus. Стойностите (mean±S.E.M.) на прага на болката (PPT) и прага на болковия толеранс (PTT) са измерени в покой или след натоварване (с и без feedback) и анализирани с Friedman ANOVA или Wilcoxon тест. Установено е, че: - стойностите на PPT и PTT се повишават при нарастване на скоростта на компресия; - субмаксималното натоварване предизвиква хипоалгезичен ефект. Тези резултати показват, че пресорната алгометрия е подходяща за измерване на чувствителността към болка на соматичната тъкан на крайниците, включително на скелетните мускули при натоварване.

Ключови думи: болклив праг; компютъризирана алгометрия, скелетни мускули; натоварване.

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2.22.

РОЛЯ НА ТЕСТОВЕ ЗА АЕРОБЕН КАПАЦИТЕТ ЗА ОБУЧЕНИЕТО ПО СПОРТ ПРИ ЗДРАВИ НЕТРЕНИРАНИ СТУДЕНТИ

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Резюме. Целта на настоящия доклад е да се представи оценяването на аеробния капацитет на здрави, нетренирани студенти като актуален проблем на спортната физиология и спортната педагогика. Анализирани са параметрите на аеробния капацитет (VO₂max, Wmax, нивата на лактат и др.), някои максимални и субмаксимални тестове и са представени аргументи за приложението на тестовете в обучението по спорт при нетренирани студенти (19-26 год). Изложените данни доказват ролята на функционалните изследвания върху аеробния капацитет за оптимизирането на учебно-тренировъчния процес при здрави, нетренирани студенти и за развитието на студентския спорт.
3.14.
ASSESSMENT OF TRAINING IN YOUNG CANOEING ATHLETES BY MUSCLE FIBRE CONDUCTION VELOCITY

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РЕЗЮМЕ. Цел на настоящото изследване е: - да се оцени нивото на тренираност чрез измерване на скоростта на провеждане по мускулни влакна (CV – conductance velocity) на осреднените потенциали на интерферентна ЕМГ при състезатели по кану-каяк (юношеска група) и при нетрениращи лица от същата възрастова група; и – да се проучат промените в скоростта в резултата на периферна мускулна умора при двете групи. Изследвани са 15 състезатели по кану-каяк (14-17 г.; 16.3 ± 0.76 г.), които са тренирали не повече от 3 пъти седмично в продължение на 2 години и 16 здрави, нетрениращи тийнейджъри на същата възраст. ЕМГ се регистрира по време на волева, изометрична супинация на предмишницата при 90° флексия при 100 %, 75 %; 50 % и 25 % от максималната волев контракция (МВК) преди, по време и след протокол за генериране на мускулна умора. Волевите контракции бяха с продължителност от 3 с при 3 повторения. Интерферентната ЕМГ се регистрираше от m. biceps brachii на дясната (доминантна) ръка с повърхностни, жичкови електроди.

Осреднените потенциали при различните групи се сравняваха с непараметричните Mann-Whitney U test и Wilcoxon match-paired test при оценка на значимостта на междугрупови и вътрегрупови разлициата, съответно. Получените резултати показваха, че: - средните стойности на CV зависят (p<0.05) от нивото на мускулната активност при състезателите по кану-каяк и не зависят при нетрениращите; - стойностите на CV са значимо по-високи при трениращите; - стойностите на CV намаляват статистически значимо след протокол за умора при 25 % и 100 % от МВК при нетрениращите и при 100 %, 75 % и 50 % от МВК при състезателите по кану-каяк. Получените данни са основание за следните предположения: - CV по мускулните влакна, като основен физиологичен параметър за функционалното състояние на скелетните мускули, може да бъде използван за предсказване на нивото на тренираност при състезатели по кану-каяк, а вероятно и при редица други видове спорт; - различията между трениращите и нетрениращите юноши могат да се обвържат с различия в рекрутiranето на двигателните единици, което води до по-ранното обособяване на големите двигателни единици и обяснява поведението им при по- интензивни натоварвания; - промените в CV при трениращите лица могат да бъдат резултат от промени в типа на активация и/или невронална и морфологична адаптация.
6.1.6.

PAIN THRESHOLD AND PAIN TOLERANCE IN DANCERS

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Differences in pain threshold (PT) and pain threshold tolerance (PIT) have been founded, depending on the types of exercise. In this study we compared a PT, poor pain threshold (PPT), powerful tolerable pain threshold (PTPT), PIT and pain rating (PR) in folk dancers and in untrained subjects, before and after graded exercise. The cardiovascular and biochemical parameters were also compared. PT, PPT, PTPT, PIT and PR were assessed with pressure algometry. The visual-analog scale (VAS; 0-100 mm) was used for PR PR before and after exercise by palpation of vastus lateralis was also determined. There was no pain during palpation, before and after loading in dancers, while in the untrained the pain level in the insertion was 1.55±0.9 mm and 0.35±0.2 mm, respectively. The PR was lower (p <0.05) for dancers before and after exercise, as compared with untrained. PPT, PTPT and PIT were increased after exercise for both groups, but the values for dancers were 1.5 times higher (p<0.05). The results of this study suggest that: 1) the dancers have higher PIT and lower PR, at rest and after exercise; 2) the regular physical activity and the graded exercise to exhaustion produce manifested hypoalgesic effects.

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6.1.11.

Oral presentations

OP-PH07 Physiology 7 - Thermoregulation and Heat Stress

CANNABINOID AND OPIOID ANTI-NOCICEPTIVE MODULATION OF MECHANICAL AND THERMAL STIMULI AFTER LIMITED MOTOR ACTIVITY

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Introduction

Exercise and limited motor activity induce hypoalgesia, including in the skeletal muscles (Dietrich, McDaniel, 2004). The aim of this study was to test the contributions of cannabinoid CB1 and opioid receptors in anti-nociceptive modulation across mechanical and thermal stimulus modalities in acute or repeated immobilization.

Methods

Nociception was measured in rats with Paw pressure or Hot plate test. The threshold measure with Paw pressure test (P-PTT) and the paw lick latency (sec) as threshold of Hot plate test (T-HPT) were assessed. For immobilization stress the animals were placed in a tube for 3 hours or for 4 days. Naloxone (opioid receptor antagonist) and AM251 (CB1 receptor inverse agonist) were administrated i.p. after the stress and before nociceptive testing. All values were expressed as mean ± SEM. One-way ANOVA was used to verify the statistical significance (p < 0.05) between the treated and control groups.
Results
Acute immobilization increased the values of P-PPT (g/cm2) and P-HPT (sec) form 116±13 to 216±19 and from 3.9±0.5 to 6.3±0.7, respectively. Naloxone (2 mg/kg) induced hyperalgesia, more pronounced for mechanical pain. AM-251 (1.25 mg/kg) reduced the values of P-PPT and P-PHT with 58±75% and 44±6% respectively. The application of naloxone and AM-251 induced a decrease of the values of P-PPT with 73±8% and for P-HPT with 59±7%. Repeated immobilization did not evoke hypoalgesia, but attenuated the hyperalgesic effect of naloxone with 12±3% for mechanical and 22±3% for thermal stimulation, and of AM-251 with 33±4% and 40±6%, respectively. Simultaneously application of AM-251 and naloxone reduced the values of the mechanical threshold with 38±5%, but of thermal with 25±4%.

Discussion
The results suggest that: - acute and chronic immobilization-induced hypoalgesia is mediated by CB1 and opioid receptors; - antimechanical pain effect is 2-2.5 fold stronger than antithermal; - the cumulative effect of opioid receptors and CB1 receptors is observed in the acute stress and in the mechanical modality of the chronic. The cross-talk between cannabinoid and opioid systems, supported by anatomical, biochemical and molecular data (Robledo et al., 2008) is revealed. The effects of the opioid system (Pencheva, Bojkova, 2004) are more pronounced in acute immobilization stress, while the cannabinoid mechanisms predominate probably after chronic stress.

References

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knee (flexion/extension; speed: 60 °/s) muscle strength, an isokinetic dynamometer (Biodex System 4 Pro) was used.

**Results.** The medial part of the GS muscle was more sensitive to pressure (PTT, kPa/cm²: 350±25-right side; 350±24-left side) as compared with the lateral (1430±18 and 390±19, respectively), but only the right lateral part was less sensitive as compared to the left one. The EIH (kPa/cm²) was maximal for the lateral part of right GS (150.2±17.39) and was more pronounced in comparison with unloaded limb (right BB: 63.5±15.81). The EIH correlated (p<0.05) to ankle muscle strength (plantar flexion 117.8±8.7 NM; r=0.77) and knee muscle strength (flexion, 121.3±6.7 NM, r=0.69; extension, 239.5±11.8 NM, r=0.54).

**Discussion.** The differences in PPT's between lateral and medial part of GS found in the present study, correspond to suggestions (Polianskis et al., 2001) for different nociceptor density in functionally diverse locations of a muscle. The EIH is not localized to the exercising body part. Central pain inhibitory mechanisms probably mediate the EIH (Koltyn, 2000; Drury et al., 2004; Pencheva and Petrova, 2005), but the greatest increase in PPT occurs in the exercising limb. The correlation between the EIH with isokinetic muscle strength found in this study suggests that the endogenous modulation of pain perception following exercise improves muscle strength.

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6.1.13.
H17 Physical Activity & Health in Special Populations

**ASSESSMENT OF AEROBIC CAPACITY AND MUSCLE FUNCTION IN HIGH SCHOOL MALE STUDENTS**

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**Introduction.** The role of the physical education teacher is to prepare proper programs of the students and to develop their fitness level, aerobic capacity and muscle strength. So, the aim of our study was: - to test the aerobic capacity and isokinetic knee and ankle muscle strength of male students, and - to assess the correlation between the isokinetic muscle strength and aerobic aptitude of the students.

**Methods.** Twenty male, high school students (age: 22.5±2.3 years, height: 178.4±13 cm, weight: 79.1±3.0 kg) performed treadmill test (Tzvetkov et al., 2008) at an initial speed of 6 km/h, an increase by 1.2 km/h every 90 s until exhaustion. To measure student's maximal ankle (plantar/dorsal flexion speed - 30, 60, 120 °/s) or knee (flexion/extension, speed - 60, 180, 300 °/s) muscle strength, an isokinetic dynamometer (Biodex System 4 Pro) was used. Body composition was assessed by electrical bioimpedance.

**Results.** The mean values±SD for the peak responses of VO2 (ml/min/kg), VE (l/min), HR (bpm) and speed (km/h) were: 48±5, 115±25, 190±8 and 11.2±0.9, respectively. The knee extensor/flexor peak torque (mean±SEM) at 60, 180 and 300 °/s was 230±12/117±6, 150±8/87±5 and 104±6/67±4 Nm respectively. The maximal isokinetic plantar/dorsal flexion torques at 30, 60 and 120 °/s were 118±9/45±4, 101±8/34±2 and 75±5/23±2 Nm respectively. A decrease (p<0.05) of the knee
flexion/extension ratios, was established. The individual muscle strength for knee extensors, knee flexors and ankle plantar flexors was significantly (p<0.05) related to the fat free mass (r=0.80). However, neither peak VO2, nor other parameters of aerobic capacity were related to the muscle strength.

**Discussion.** Isokinetic muscle strength and maximal aerobic capacity are independent of each other in untrained male students. However, the values for isokinetic knee and ankle muscle strength, which are in agreement with those of other researchers (Dvir, 1995, Schiltz el al., 2009), are accompanied with average aerobic capacity and low pulmonary ventilation. So, the relevant physical education programs have to be recommended in order to increase cardiopulmonary endurance and aerobic power of the students, although the majority of them prefers isometric training. Because of the muscle imbalance between knee flexors/extensors, special exercise, to increase the strength of hamstrig is also appropriate.

**References**

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2.12.

Стабилност на олигонуклеотиди, кодиращи [Met]$^5$-енкефалин

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Резюме: По чисто математичен път са формулирани критерии за устойчивост (изменичност) на генетичната информация заложена в първичната структура на нуклеиновите киселини. В тази работа се прави опит за оценка на тези критерии чрез съпоставянето им със свойства на олигонуклеотиди получени чрез молекулно-механични изчисления. За получаването на адекватни молекулни модели на олигонуклеотиди, кодиращи структурата на [Met]$^5$-енкефалин, е използван програмния пакет Hyperchem 7.0 и силовото поле AMBER 96. Оптимизирана е структурата на моделите и са изчисленi техните потенциални енергии. Сравнението на изчислените на този начин енергии дава възможност да се проверят изведените критерии и да се оценi еволюционния потенциал на изследваните олигонуклеотиди. От получените резултати можат да се направят следният изводи: - Оптимизираните структури на намерените в природата нуклеотиди последователности заемат средно положение по енергия. Това предполага средно ниво на устойчивост на генетичния код заложен в тях, както и възможност за еволюционни промени; - Генерираните чрез съвременния генетичен код нуклеотидни секвенции на [Met]$^5$-енкефалин са доста различни по енергия, но като цяло са с по-висока енергия от тази на теоретичните кодове. Това предполага по-малка устойчивост на генетичния код, заложен в тях и възможност за мутации.

Keywords: устойчивост на генетичната информация, олигонуклеотиди, молекулна механика.

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2.20.

Stability of oligonucleotides as a criterion for resistance of the genetic code

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Abstract

Different criteria for gene stability have been derived mathematically. To our knowledge, there has been no experimental or theoretical support of these criteria so far. This study presents the results of an attempt to evaluate the mathematical criteria for gene stability based on the potential energy of the coded oligonucleotides. This energy strongly depends on the code considered: contemporary (CGC) or theoretical genetic code, because nucleotides sequences coding particular chains define specific internucleotide interactions. Mathematical modeling of CGC, algorithm for solving of the discrete optimization problems and fully parametrized computational chemistry methods are employed. Methionine enkephalin coding oligonucleotides are used as model systems. Parameters of the mathematically generated and natural oligonucleotides, including the potential energy, were calculated and analyzed. Additional search in biological databases for the occurrence of the nucleotide sequences in
biological species was also undertaken. Optimal oligonucleotide sequences for various codes, including CGC, were established and compared in various species. The resistance of the code against point mutations was evaluated. The results obtained make it feasible to suggest that:

(i) the computational chemistry methods together with appropriate mathematical modeling are useful tools for the investigation of genetic code stability; (ii) the lowest species reveal the highest values of the "average potential energy", while the lowest values are typical for humans and mammals; thus the methodology presented allows investigations on the evolution of the biological species.

Keywords: contemporary genetic code; potential energy; resistance; mathematical modeling; discrete optimization

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Explicit description of the set of all theoretical genetic codes

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An aspect of the evolution of the genetic code is to minimize the number of errors during transcription and translation. In the pertinent literature this problem is analyzed by comparing of the genetic code and the set of theoretical codes generated randomly. In this study we present an explicit description of the set of all theoretical genetic codes as a convex polytope and prove that the characteristic vectors of these codes are vertices of this polytope. Thus, the modelling, obtained by us, sheds a new light on the mathematical analysis of the optimality of the genetic code, and allows new classes of optimization problems to be formulated and investigated, including the minimization of the errors.

At the same time the natural genetic code reveals the maximum resistance towards the translation errors. The polytope description obtained by us, gives a possibility to analyze the properties of all theoretical genetic codes, to characterize their translation errors and to compare them with those of the contemporary genetic code. Our calculations confirm that the classical genetic code is closed to the optimal one, with respect to point mutations. However, the further analysis does not give much information about the mechanisms of this evolution with respect to the minimization of the mutation errors.

Key Words: optimization, genetic code, mutations

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The Slope as a Parameter in Fitting of Concentration-Response Relations

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Abstract: To elucidate the role of the slope as a parameter in fitting of concentration-response curves in the quantitative pharmacology, four enkephalin analogues were tested for their effectiveness in depressing electrically evoked contractions in some isolated tissues, containing specific receptors for the enkephalins. The experimentally obtained inhibitory effects in a form of curves and two computer's simulated data points, were approximated with both - two-parametric hyperbolic function (TPH: E - 3o[AJ]/(JAM+J[A])) and three parametric hyperbolic-function (THPH: E = adAf /([Aw]* (A))): A - opioid agonist: [A] - concentration of the agonist: E - the measured inhibitory effect; a₀ -
the asymptote ($a_0 = \xi_j$ where $E^*$ maximum response of A and $E^\wedge$ potential response of the tissue): $\ln A - \ln J$ a location parameter, which is the concentration of an agonist, which produces 0.5 $e^*$; and $p$ the slope parameter The obtained results suggest that: (i) the approximation with TPH and THPH is appropriate when the value of slope belongs to the interval (0.70, 1.30). additional procedure's have to be applied for these cases when the differences between theoretical $E^{m}_{m}$ and experimental $E^{A}_{m}$ are significant: (ii) when the value of slope belongs to the interval (0.00, 0.60) the filling with THPH is better than TPH; (Hi) the value of slope is less than 0.30 for partial agonists with $E^\wedge$ tower than 0.30 and low efficacy, the fitting functions for these cases could be THPH or straight line, which depends from the distribution of the experimental data points The dissociation constant $K_A$ and relative efficacy $e_{m;i}$ were determined using explicit formulas derived by fitting of the data points with TPH.

**Keywords:** Concentration-response curves; Affinity; Efficacy; Slope; Partial agonist.