Symposium

Pharmacology of addiction

Pharmacological detoxification treatment of addicts. What’s new and clinically useful?

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It’s confusing what the unitary concept of detoxification is; clinically, the phenomenon squeezed between emergency medicine and long-term complex therapies of dependency. It’s partially the physiological as well as medicinal removal of toxic substances from a human being, with limited usefulness circumscribed with potentially dangerous symptoms of withdrawal syndrome. Newly published the DSM-5 draft proposed important physiological and diagnostic revisions; i.e. the elimination of current categories of substance abuse and substance dependence and replacing them with a new group category of addiction and related disorders. This will include substance use disorders, with each drug identified as a single category. This starts with premise that the tolerance and withdrawal experiences could be normal responses to prescribed medications affecting the central nervous system. The miscellaneous discontinuation syndromes (separate from substance use disorders), including effects of two categories of antidepressants – tricyclic and selective serotonin reuptake inhibitors, manifest themselves as the most puzzling idea of DSM-5. These phenomena occur when an individual is not ‘addicted’ in the sense that one engages in compulsive drug-seeking behavior but rather abruptly stops a medication and experiences unpleasant, sometimes serious withdrawal symptoms. Moreover, there is an overlapping concept of behavioral addictions, as gambling disorder, internet addiction, etc. The general idea of detoxification, during which an organism returns to homeostasis after long-term use of an addictive substance, is too conventional when faced with those proposals. The decontamination from poison ingestion and the use of antidotes as well as techniques such as dialysis are too narrow concepts as for any long-term treatment-oriented goals, detoxification is not a dependence treatment or even can be contradictory for that, as some psychotherapeutic approaches are focused on and utilize withdrawal experiences.
Drugs modulating memory: new perspectives in the treatment of addiction

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It is a common observation that passive exposure of drug addicts to discrete drug-associated cues can evoke intense craving and increase the risk of relapse to compulsive drug-seeking behavior. A similar picture emerged from studies on reactivity to food-associated cues in patients with eating disorders.

A brief re-exposure to the drug-associated context or discrete cues is associated with reactivation of memory of drug seeking behavior. Recent studies indicate that the retrieved memory once again became labile and transiently sensitive to disruptive effects of amnestic agents.

The preclinical experiments revealed that electroconvulsive shock or systemic drug administration given after memory reactivation can cause amnesia for the original learning. For example, Nader et al. [Nature, 2000] injected a protein-synthesis inhibitor, anisomycin, into the amygdala following the presentation of an auditory cue, which had been paired with shock. They reported that this treatment reduced the amount of freezing to that cue when the subject was tested a second time.

There are also clinical studies indicating that during reconsolidation phase old memory traces can be disrupted. Rubin [Can Psychiatr Assoc J, 1976] showed that individuals, suffering from obsessive-compulsive disorder or hallucinations, who received electroconvulsive shock (ECS) after being prompted to focus on their obsession, improved significantly. There was no such effect when the ECS was given to the unconscious patients.

These findings suggest that reconsolidation phase may be a potential therapeutic target in the treatment of various psychiatric disorders. On a theoretical ground, amnestic agents like protein synthesis inhibitors, or glutamate receptors antagonists could be considered as a new class of therapeutics for those psychiatric disorders in which addiction-like patterns of compulsive behavior are induced by discrete environmental cues. More specifically, these drugs could be considered as add-on medications to treatment approaches based on the cue exposure paradigm.

Changes in glutamate-catecholamine interactions underlie persistence of drug-conditioned behaviors

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Addictive drugs hijack mechanisms of learning and memory that normally underlie reinforcement of natural rewards and induce maladaptations in the interactions between catecholamine and glutamate signaling. To define the behavioral roles of glutamate-dependent plasticity in drug-induced behaviors we have generated Cre/loxP transgenic mouse models with targeted mutations of glutamate receptors restricted to discrete parts of the catecholamine systems. The Cre recombinase was expressed under the control of neuron type...
specific promoters derived from either the dopamine transporter (dopaminergic cells), dopamine D1 receptor (dopaminoceptive cells) or dopamine beta-hydroxylase (noradrenergic cells). The targeted ('floxed') gene was the NR1 subunit of the NMDA receptor, which is essential for forming of functional channels. We found that loss of functional NMDA receptors in dopaminergic or dopaminoceptive neurons abolished reinstatement of drug-conditioned place preference, but did not affect psychomotor sensitization. Conversely, loss of NMDA receptors located on noradrenergic neurons abolished opiate-induced psychomotor sensitization, while having no effect on conditioned place preference. These results reveal respective contributions of the catecholamine-glutamate interactions in the persistence of drug induced behaviors, and define targets for design of improved pharmacotherapies.

The serotonin (5-HT)_{2A} and 5-HT_{2C} receptors in nicotine dependence

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Nicotine is one of the most common and legal addictive substance. As current strategies approved for smoking cessation lack significant efficacy, the researchers are still trying to develop more effective treatments [Cryan et al., Drug Discov Today, 2003]. Although the dopaminergic system is associated with nicotine dependence [Balfour, Curr Drug Targets CNS Neurol Disord, 2002], recent data point to a role of serotonin (5-HT) and its receptors in the effects of nicotine [Fletcher et al., Prog Brain Res, 2008]. Our studies demonstrate that 5-HT_{2A} and 5-HT_{2C} receptors alter some of the behavioral effects of nicotine. The pharmacological blockade attenuates nicotine sensitization, conditioned locomotion and depression-like behavior during nicotine withdrawal. Conversely, stimulation of these receptors enhances nicotine sensitization and conditioned locomotion. Activation of 5-HT_{2C} receptors diminishes the expression of nicotine sensitization, conditioned locomotion and nicotine withdrawal symptoms. Additionally, autoradiographic analysis revealed that chronic nicotine treatment alters the radioligand binding to 5-HT_{2} receptors depending on animal model and brain regions. Thus, \[^3H\]ketanserin binding to 5-HT_{2A} receptors was decreased in the striatum in nicotine-sensitized rats and in animals exposed to the environmental stimulus while increases were observed in the dentate gyrus and ventral tegmental area during nicotine withdrawal. The 5-HT_{2C} receptor radioligand \[^3H\]mesulergine evoked a decrease in binding to the receptors in the prefrontal cortex in nicotine-sensitized rats and in the ventral dentate gyrus or thalamic nuclei during nicotine withdrawal.

Concluding, tonic activation of 5-HT_{2A} receptors and pharmacological stimulation of 5-HT_{2C} receptors play an essential inhibitory role in behavioral effects of nicotine. Obtained data show a new direction in the search for efficient anti-addictive drugs and the possibility of using 5-HT_{2A} receptor antagonists and 5-HT_{2C} receptor agonists in the pharmacotherapy of nicotine dependence.
Symposium

Pharmacology of adipose tissue

Lipophilic statins increase endogenous H$_2$S formation in perivascular adipose tissue by inducing local coenzyme Q deficiency

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Hydrogen sulfide (H$_2$S), apart from nitric oxide and carbon monoxide, is the third endogenously generated “gasotransmitter”. H$_2$S is synthesized in the cytosol by cystathionine $\beta$-synthase (CBS) and cystathionine $\gamma$-lyase (CSE), and is oxidized in mitochondria. Recently, it has been demonstrated that H$_2$S produced in perivascular adipose tissue dilates blood vessels by activating K$_{ATP}$ channels in smooth muscle cells [Fang L et al., J Hypertens 27:2174, 2009]. We examined the effect of statins on H$_2$S production in perivascular adipose tissue (PAT) in the rat. Pravastatin or atorvastatin were administered for 3 weeks at doses of 40 and 20 mg/kg/day, respectively. Formation of H$_2$S from cysteine in PAT homogenates in vitro was 64% higher in atorvastatin-treated rats, whereas in pravastatin-treated animals did not differ from control. H$_2$S formation in isolated cytosolic fraction (without mitochondria) was higher than in mitochondria-containing homogenates, and was similar in all groups. In addition, CSE activity and mRNA levels were similar in PAT of control and statin-treated rats. In contrast, the rate of oxidation of exogenous H$_2$S by isolated liver mitochondria was lower in statin-treated than in control rats. However, activity of the rate-limiting enzyme of H$_2$S oxidation, sulfide:quinone oxidoreductase, was similar in control and statin-treated animals when exogenous coenzyme Q was added to the incubation medium. Although both statins decreased hepatic and plasma coenzyme Q$_9$, only atorvastatin reduced CoQ$_9$ concentration in PAT. Supplementation of exogenous coenzyme Q prevents atorvastatin-induced increase in H$_2$S production in the PAT, but had no effect on H$_2$S formation in rats not receiving statins. These results indicate that lipophilic atorvastatin, by inducing local coenzyme Q depletion, inhibits mitochondrial H$_2$S oxidation and increases its net production in PAT. Since PAT-derived H$_2$S decreases vascular tone, this effect is the first beneficial consequence of statin-induced CoQ deficiency.
Leptin receptor antagonists: preparation and use in research and medicine

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Leptin is a pleotropic hormone that acts both centrally and peripherally. It exhibits both positive effects such as regulation of energy metabolism, reproduction, immune response as well as negative actions, such as enhancement of undesired immune responses in autoimmune diseases, tumorigenesis, elevated blood pressure and cardiovascular pathologies. We prepared leptin mutants by alanine mutagenesis of amino acids LDF (39–41). Those mutations abolished the agonistic activity of human, ovine, rat and mouse leptins and converted them into potent antagonists that bind to LEPR with affinity similar to the wild-type hormone and specifically inhibit leptin action in several leptin-responsive in vitro bioassays. To prolong and enhance the in vivo action of leptin antagonists we increased their molecular mass and hydrodynamic volume by pegylation. Administration of the pegylated mouse leptin antagonist (PEG-MLA) to mice resulted in a rapid, significant and fully reversible weight gain, due to enhanced appetite and increased food consumption. The mechanism of severe central leptin deficiency resulted mainly from inhibition of leptin transport across the blood-brain barrier and limited accumulation of PEG-MLA in the hypothalamic region. Those findings were evidenced by following-up the distribution of radio-labeled or by Alexa Fluor®680-conjugated PEG-MLA and MLA in the body. MLA and PEG-MLA were also tested in vivo as blockers of experimental leptin-enhanced mice models of T-cell dependent and non-dependent acute hepatitis and chronic liver fibrosis. Antagonists exhibited an anti-inflammatory and anti-fibrotic activity and improved survival. To enhance the effectiveness of MLA we used random mutagenesis followed by selection of high-affinity mutants by yeast surface display and subsequently developed a novel mutant of MLA with 10-fold increased inhibitory activity. In conclusion we introduced a novel compound that induces central and peripheral leptin deficiency, is useful in exploring the role of leptin in metabolic and immune processes, and could eventually serve as a therapeutic modality for treatment of leptin-enhanced autoimmune diseases and cachexia.

Leptin as an endogenous pro-hypertrophic factor contributing to myocardial remodelling and heart failure

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Leptin is a member of the adipokine family of peptides produced primarily in adipocytes. This 16 kDa pleiotropic hormone exerts a multiplicity of biological effects although its primarily role is the central regulation of appetite and energy expenditure. Leptin is also produced in cardiac myocytes and there is now increasing evidence that leptin, acting via its receptors expressed in the cardiac myocyte, exerts a direct pro-hypertrophic effect thus contributing to myocardial remodelling and heart failure. Addition of physiological concentrations of leptin to cultured ventricular myocytes results in marked hypertrophic effects. Moreover, leptin synthesis in cardiac myocytes is stimulated by both angiotensin II and endothelin-1 and the pro-hypertrophic effect of both agents is leptin-dependent. The mechanisms underlying the pro-hypertrophic effect of leptin appear to be complex and likely involve multiple cell signalling mecha-
nisms possibly acting in concert. A potentially critical pathway mediating the pro-hypertrophic effect of leptin is the RhoA cascade whose activation ultimately results in alteration in actin dynamics as manifested by a decreased G/F actin ratio and which further results in the selective translocation of p38 MAPK into nuclei followed by activation of transcriptional factors. The contribution of endogenous leptin to cardiac pathology is further demonstrated by the ability of leptin receptor blockade to significantly reduce both hypertrophy and remodelling in the post-infarcted myocardium as well as to improve left ventricular function. Another adipokine, adiponectin is also produced by cardiac myocytes which also express adiponectin receptors. However, in contrast to leptin, adiponectin exerts antihypertrophic effects. Indeed, the pro-hypertrophic effects of leptin are markedly reduced by adiponectin. The leptin/adiponectin ratio may therefore determine the nature of the response to the hypertrophic effect of leptin.

Effect of hypolipidemic drugs on adipokines release by visceral adipose tissue and isolated adipocytes of patients with atherogenic dyslipidemia

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Background: The human adipose tissue is distributed in two general ways: visceral and subcutaneous. Both are not only the energy storage but also very active endocrine organ releasing adipokines and cytokines. There are adipokines considered as “beneficial”, like adiponectin – connected with the positive metabolic feedback, and “detrimental” – resistin, visfatin – responsible for the insulin resistance in obesity.

Design and methods: Isolated adipocytes obtained from visceral and subcutaneous adipose tissue of 19 patients with mixed dyslipidemia were incubated in vitro in the presence of atorvastatin and/or fenofibric acid. Adipocytes from 19 subjects with normal lipid profile were cultured as a reference.

Results: Compared with cells of patients with normal lipid profile, adipocytes of dyslipidemic patients secreted distinct amounts of adiponectin, leptin, resistin. In visceral adipocytes of patients with mixed dyslipidemia, both drugs administered alone increased adiponectin secretion and reduced resistin release. A combined treatment changed the release of all studied markers even more. In adipocytes from subcutaneous adipose tissue, combined treatment with both drugs increased adiponectin release and reduced resistin secretion. Atorvastatin administered alone increased adiponectin release. Adipokine secretion by adipocytes of normolipidemic subjects was only slightly affected, mainly when adipocytes were treated with both agents. In addition, the proinflammatory cytokines profile were also studied. Adipocytes derived from dyslipidemic subjects secreted more TNF-α, interleukin 6 and PAI-1. In dyslipidemic and normolipidemic subjects visceral tissue secreted less TNF-α and PAI-1 than subcutaneous one. Combined treatment reduced TNF-α and interleukin-6 release, while fenofibric acid alone decreased only TNF-α release. All considered treatments changed the PAI-1 release.

Conclusions: The amount and pattern of adipokine release differs between subjects with and without lipid abnormalities, and between adipocytes obtained from visceral and subcutaneous adipose tissue. The hypolipidemic drugs, statins and fibrates, affect the release of adipokines by human adipocytes. The effect increases with the drug dose and is the most prominent when this two drugs are combined. This impact may contribute in the clinical effectiveness of combined therapy in the prevention and treatment of dyslipidemia-related cardiovascular and metabolic disorders.
Bivalirudin and Fondaparinux – why do they displace older anticoagulants in new guidelines in cardiology?

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Appropriate anticoagulant treatment, together with antiplatelet co-therapy, plays an important role in effective treatment of patients with range of cardiovascular syndromes such as acute coronary syndrome (ACS), pulmonary and peripheral thrombosis, atrial fibrillation, stroke, vascular interventions, cardiosurgery. Current guidelines generally recommend anticoagulant strategy; however, there are some differences in indications, classes of recommendation and levels of evidence of anticoagulants. There is a wide range of anticoagulants, from the oldest therapeutic agent – unfractionated heparin (UFH) – through low-molecular-weight heparin (LMWH), to selective inhibitors of factor Xa such as fondaparinux and direct inhibitors of thrombin such as bivalirudin.

Fondaparinux is a selective inhibitor of factor Xa. This is a synthetic pentasacharide modelled after the antithrombin-binding sequence of UFH. Fondaparinux is administered subcutaneously, has no antigenicity, does not cross the placenta and heparin-induced thrombocytopenia (HIT) antibodies. It is eliminated mainly by the renal route. Clinical trials showed decreased bleeding complications versus UFH or LMWH. One of the disadvantages is that it is difficult to monitor. Also thrombosis on catheters has been noted when using only fondaparinux in the cath lab so it is not recommended as the sole anticoagulant to support percutaneous coronary intervention.

Bivalirudin is a direct thrombin inhibitor. Inactivating both fibrin-bound and fluid-phase factor-IIa, bivalirudin inhibits thrombin-induced fibrinogen to fibrin conversion. Linear pharmacokinetics, high specificity to thrombin and high correlation between dose administered intravenously and APPT make the anticoagulant effect predictable and easy to monitor. The main advantage of bivalirudin is its safety. Bivalirudin appears to be more effective than UFH in risk reduction of adverse cardiac events and bleeding, and safer than UFH in combination with GP IIb/IIIa inhibitors among patients undergoing PCI in ACS. Currently bivalirudin is recommended for urgent and elective PCI and treatment of HIT complicated by thrombosis.

This lecture presents newly available anticoagulants and summarizes current guidelines on anticoagulation therapy in various cardiovascular syndromes.
Treatment of pulmonary embolism according to current guidelines

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Pulmonary embolism (PE) is a common cardiovascular syndrome, very often life threatening, but also under-diagnosed in clinical practice. On the other hand early diagnosis of PE is crucial in success of treatment. Diagnosis of PE is based on clinical findings in combination with laboratory tests and imaging studies. Treatment of PE is typically with anticoagulant medication, including heparin and warfarin. Severe cases may require thrombolysis with drugs such as tissue plasminogen activator (tPA) or may require surgical intervention via pulmonary thrombectomy. Thrombolytic therapy is the first-line treatment in patients with high-risk PE presenting with cardiogenic shock and/or persistent hypotension. Routine use of thrombolysis in non-high risk patients is not recommended, but may be considered in selected patients with intermediate-risk PE. Anticoagulation with unfractionated heparin, low-molecular-weight heparin or fondaparinux should be initiated without delay in patients with confirmed PE and those with a high or intermediate clinical probability of PE. Specific problems in treatment of PE includes: pregnancy, malignancy, right heart thrombi, heparin-induced thrombocytopenia, chronic thromboembolic pulmonary hypertension and non-thrombotic PE. In 2009 European Society of Cardiology published new guidelines on the diagnosis and management of acute PE. The aim of this presentation is to present recommended diagnostic and pharmacological management in PE based on current guidelines and selected medical publications.

New oral antithrombotic agents – where we are?

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Two new oral antithrombotic agents have been registered recently: dabigatran etexilate (Pradaxa®) and rivaroxaban (Xarelto®). They are the fixed-dose oral anticoagulants licensed for the prevention of venous thromboembolism in adults who have undergone or are undergoing elective replacement of the hip or knee joints. However the trials comparing those agents with warfarin are available (RELY for dabigatran) or awaited late in 2010 (ROCKET AF for rivaroxaban). There are some considerable limitations in the published evidence for both drugs, which have not been directly compared with each other, or with anticoagulants other than enoxaparin in elective major lower limb orthopaedic surgery. However, the simplicity of fixed daily oral dosing without monitoring and potential cost-effectiveness of dabigatran etexilate and rivaroxaban are attractive options when patients are either not willing or not capable of self-administration of a subcutaneous alternative. It is very much awaited alternative for growing number of patients with atrial fibrillation. The long, unhappy reign of warfarin (or – much often in Poland – acenokumarol) is probably drawing to a close. The published in 2009 RELY trial may have finally identified a candidate, dabigatran, an oral direct thrombin inhibitor that could one day replace warfarin in atrial fibrillation. Unlike warfarin, dabigatran does not require dose adjustments or anticoagulation monitoring. RELY randomized 18,113 AF patients to either one of two fixed doses of dabigatran (110 mg or 150 mg twice daily) or warfarin. The RELY investigators concluded that “compared with warfarin, the 110-mg dose of dabiga-
tran was associated with similar rates of stroke and systemic embolism and lower rates of major hemorrhage; the 150-mg dose of dabigatran was associated with lower rates of stroke and systemic embolism but with a similar rate of major hemorrhage”. The primary endpoint was stroke or systemic embolism. The investigators found no evidence of liver toxicity, which was the downfall of ximelagatran, the previous warfarin replacement candidate. The study was designed to evaluate whether either of two doses of dabigatran were non-inferior to warfarin (i.e., at least as good as warfarin). The results show, however, that the higher dose of dabigatran, 150 mg twice daily, significantly reduces the risk of stroke by 34% compared to warfarin. The lower dose, 110 mg twice daily, had a similar effect to warfarin in the prevention of stroke, but with significantly less major bleeding. Several other new drugs have been recently studied to see if they could replace warfarin. These are also: rivaroxaban, apixaban, betrixaban – all discussed in the short lecture.

Heparin and low-molecular-weight heparin – actual place in therapy, what we have learned in previous years in cardiology?

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Anticoagulant use is recommended for thrombotic event prevention in many cardiovascular diseases, including stroke prevention in atrial fibrillation, treatment and secondary prevention of acute coronary syndrome. Current parenteral anticoagulants include unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs) and fondaparinux. The cornerstone of acute coronary syndromes (ACS) management includes antiplatelet agents, antithrombin therapy, and fibrinolytic or invasive revascularization. Evidence-based guidelines for the management of ACS identify a central role for UFH or LMWH. UFH has been widely studied and used in patients with unstable angina pectoris (UAP), non–ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI). Its use in these diagnoses is a class Ia indication, and its efficacy has been shown in early trials to reduce myocardial infarction (MI) over β blockers and aspirin and then as adjunctive therapy to aspirin. In patients with acute myocardial infarction, several studies have shown that LMWHs represent an effective alternative to UFH as an adjunct to thrombolytic therapy and are not associated with an increased risk of major bleeding. In patients with unstable angina and NSTEMI many data support the use of the LMWH enoxaparin to reduce cardiovascular events and death. LMWHs also appear more effective than unfractionated heparin in reducing the composite end point of acute MI, recurrent ischemia, or death in patients with STEMI, and can also be used effectively in patients undergoing thrombolysis reperfusion and percutaneous coronary intervention. It has also been shown that the LMWH enoxaparin significantly reduces the risk of cardiovascular events, compared with UFH, whereas other trials have shown that the combination of enoxaparin and a glycoprotein IIb/IIIa antagonist is not associated with an excess risk of bleeding. Patients with acute coronary syndrome usually receive unfractionated heparin or a LMWH on hospital admission, both exhibit similar efficacy in reducing mortality and myocardial infarction rates; however, LMWHs may have a better safety profile and do not require routine coagulation monitoring. However, the various LMWH preparations should not be used interchangeably. Of the several LMWH agents that have been studied in large clinical trials, including enoxaparin, dalteparin, and nadroparin, not all have shown better efficacy than UFH. Enoxaparin is the only LMWH compound to have demonstrated sustained clinical and economic benefits in comparison with UFH in the management of unstable angina/NSTEMI. Each LMWH is a pleiotropic biological agent with a unique chemical, biochemical, biophysical, and bio-
logical profile, and it displays a unique pharmacodynamic and pharmacokinetic profile. As a result, LMWHs are not equipotent in preclinical assays or equivalent in terms of their clinical efficacy and safety. Anticoagulants are also widely used for preventing VTE in a broad range of surgical and medical patients. Surveys have consistently shown that the most widely used agents are LMWHs, UFH, and vitamin K antagonists such as warfarin. LMWHs are replacing UFH for therapeutic anticoagulation owing to a number of advantages, including a more predictable pharmacokinetic profile and their ease of use. The 2008 guidelines of the American College of Chest Physicians (ACCP) recommend LMWHs, UFH, or fondaparinux as VTE prophylaxis for surgical and acutely ill medical patients.

Combined antiplatelet-anticoagulant treatment in cardiology – actual guidelines

Marek Postuła

Antiplatelet therapy is the cornerstone for both primary and secondary prevention therapies for ischemic events resulting from coronary atherosclerotic disease. Dual antiplatelet therapy (aspirin plus a thienopyridine, usually clopidogrel) has assumed a central role in the treatment of acute coronary syndromes and after coronary stent deployment. In patients with acute MI, the immediate administration of aspirin (ASA) has been shown since the 1980s to lower the rate of periprocedural MI and subsequently has become both a quality-of-care metric and a Class I indication in practice guidelines (American College of Cardiology/American Heart Association/Society for Cardiovascular Angiography and Interventions [ACC/AHA/SCAI] as well as the European Society of Cardiology [ESC]). These guidelines recommend prolonged dual antiplatelet therapy for at least 12 months after placement of drug-eluting stents (DES). Evidence-based guidelines provide several indications for oral vitamin K antagonist (VKA) administration, including atrial fibrillation, mechanical heart valve replacement, venous thromboembolism, and patients with one or more cardioembolic events. Despite proven benefit in these well-studied patient populations, emerging evidence suggests that VKAs are underused in clinical practice, particularly among those patients at greatest risk of thrombosis-related events. In addition to antiplatelet therapy, anticoagulant therapy might be indicated for stroke prevention in a variety of conditions that include atrial fibrillation, profound left ventricular dysfunction, and after mechanical prosthetic heart valve replacement. This is often due to a perceived prohibitive risk of serious or lifethreatening hemorrhagic complications. This perception is extrapolated and potentially amplified among patients with acute coronary syndromes (ACS) who undergo coronary arterial stenting and have a requisite need for dual antiplatelet therapy with aspirin and clopidogrel, in addition to a concomitant indication for anticoagulant therapy. Indeed, “triple anticoagulation” may represent a particularly unattractive option for many practicing clinicians. In the absence of data derived from randomized clinical trials or large-scale registries, the management of patients with concomitant indications for VKAs and dual platelet-directed therapy remains unclear, likely resulting in variation in physician practices. The optimal dose of ASA for acute and long-term treatment is less well-established. Aspirin-dosing regimen has important implications for bleeding, particularly in patients receiving “triple therapy” (2 antiplatelet agents plus warfarin). Bleeding risks have become more problematic with the advent of widespread and prolonged therapy with the combination of ASA and a thienopyridine. Dual antiplatelet therapy provides incremental platelet inhibition (compared with either agent alone) and more effective suppression of adverse ischemic events and has been studied in the settings of medical therapy and PCI as
well as in stroke prevention and treatment. Warfarin has also been evaluated in patients with recent MI, but combining warfarin with even a single antiplatelet agent makes bleeding a more prominent concern. Unfortunately, there is very limited information regarding patients treated with triple therapy, who present significant clinical challenges because of the imperative to balance bleeding risks against risks entailed in stopping 1 of the 3 therapies. Discontinuation of warfarin might increase the potential for stroke, whereas discontinuation of clopidogrel might result in increased risk for stent thrombosis; both events are associated with significant morbidity and mortality. Antithrombotic strategies adopted in patients who require oral anticoagulation as well as antiplatelet therapy can vary and are often left to the judgment of the attending physician.
Epilepsy and antiepileptic drugs

Management of drug-resistant epilepsy – clinical data

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Epilepsy is a common and devastating neurological disorder. In many patients with epilepsy seizures may be properly controlled with currently available antiepileptic drugs (AEDs). Around 30% of epileptic are not seizure free despite administration of AEDs in optimal doses. This is so-called drug-resistant epilepsy.

A minority of patients not responding to first-line AEDs may be rendered seizure-free with the help of newer medical treatments available in the last decade, or their combinations. Other therapeutic methods including epilepsy surgery, vagus nerve stimulation or ketogenic diet are regarded as effective alternative forms of therapy for selected patients with intractable partial epilepsy.

Clinical studies performed up till present on this issue are limited. This was a reason for presenting own observations on this particularly important therapeutic problem. The treatment results of 360 patients (males – 192, females – 168) with epilepsy who in a period of twenty years (from March 1988 to March 2008) sought assistance in Neurological Practice have been analyzed. The group aged from 18 to 78 years (mean: 62.5 yrs) were treated with monotherapy (170 patients), sequential monotherapy (69 patients), or with polytherapy (121 patients). Polytherapy data indicate that more than a 50% reduction in seizure frequency was observed in patients given combinations of carbamazepine (or valproate) + topiramate or oxcarbazepine + topiramate. Also, considerable reductions in seizure frequency were noted when combinations of valproate + gabapentin (or oxcarbazepine) were applied. Interestingly, when carbamazepine was combined with lamotrigine, an increased seizure frequency was observed.

The clinical observations, although very limited, seem correlated with experimental data on combined treatment of epilepsy.
Therapy of drug-resistant epilepsy – experimental clues

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Circa 70% of epileptic patients may be sufficiently controlled with antiepileptic drugs (AEDs). The remaining 30% of patients are not seizure-free which is mainly due to the AED-resistance. Patients with intractable epilepsy are frequently prescribed combinations of AEDs and yet only some of them can benefit from this therapeutic option. A possibility arises that the limited efficacy of AED combinations may result from not following experimental clues which clearly indicate which drug combinations exert anticonvulsant synergy and adverse antagonism.

The results obtained from experimental models of epilepsy (mainly electroconvulsions and pentetrazol-induced seizures in mice, representative of human tonic-clonic or partial seizures and myoclonic convulsions, respectively) may be divided in two main groups: these comprising combinations of an classic AED (carbamazepine, phenobarbital, phenytoin, valproate) with a newer one and those among newer AEDs only. Some older data provide evidence on the combinations of classic AEDs. As regards the first group, the best combinations are those comprising gabapentin with classic AEDs or valproate + lamotrigine (retigabine or topiramate) and carbamazepine + topiramate. The recently characterized second group of interactions between newer AEDs points especially to topiramate + lamotrigine (or gabapentin, levetiracetam, and oxcarbazepine) and levetiracetam + oxcarbazepine as very promising AED combinations. Also, combinations of gabapentin + topiramate (or vigabatrin, levetiracetam) may be regarded as beneficial from the experimental point of view. In contrast, a combination of carbamazepine with lamotrigine results in an anticonvulsant antagonism and neurotoxic synergy which renders this particular combination very hazardous to the degree the experimental data may be transferred to clinical conditions. Also, antagonism is still evident when carbamazepine is replaced with oxcarbazepine.

A possibility may be taken into consideration that the clinical results of the combination therapy of epilepsy could be improved when rational polytherapy is applied.

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Synaptic vesicle protein 2A (SV2A) mediates anticonvulsant effects of levetiracetam in mice

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Synaptic vesicle protein 2A has been identified as a binding target for the antiepileptic drug levetiracetam (Keppra®). This protein is ubiquitously expressed in the brain and has been demonstrated to modulate synaptic release, but its function is not fully characterized and thus the specific mechanism by which SV2A binding leads to seizure protection has not been fully elucidated.

Experimental data provided evidence for a strong functional correlation between SV2A binding affinity and anticonvulsant potency. Such correlation had been initially observed in the mouse audiogenic seizure model and more recently it was also confirmed in different models of both generalized and partial epilepsy. Further evidence indicating the role of SV2A in mediation of the anticonvulsant effects of leveti-
racetam has been obtained with transgenic animals. SV2A (–/–) mice develop severe seizure phenotype shortly after birth and do not survive beyond 2–3 weeks. SV2A (+/–) mice develop normally after birth, but have increased seizure susceptibility and are more prone to epileptogenesis. A reduced seizure threshold of SV2A (+/–) mice was observed in pilocarpine, kainic acid, pentylenetetrazol and 6 Hz models, but not in maximal electroshock seizure model, which mirrors the protective effects of levetiracetam in these models. SV2A (+/–) mice displayed accelerated epileptogenesis in amygdala and corneal kindling models, while levetiracetam has the opposite effect in these models. Anticonvulsant efficacy of levetiracetam, defined as its ability to increase seizure threshold for 6 Hz electrical stimulation, was significantly reduced (approx. 50%) in the SV2A (+/–) mice, consistently with reduced binding to SV2A in these mice. In contrast, valproate, which does not bind to SV2A, produced the same anticonvulsant effect in both SV2A (+/+ ) and SV2A (+/–) mice.

In conclusion, several lines of evidence indicate that SV2A protein is the main target for the anticonvulsant action of levetiracetam: 1) SV2A is the unique binding site and plays an important role in synaptic vesicle function, 2) SV2A affinity-potency correlations have been demonstrated in several models of partial and generalized epilepsy, 3) increased seizure vulnerability and accelerated epileptogenesis in mice lacking SV2A and finally 4) reduced anticonvulsant activity of levetiracetam in SV2A deficient mice.

Epileptogenesis in the immature brain – possible therapeutic target?

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Epidemiological studies indicate that about 0.8% of the world population has epilepsy and that in approximately 50% of patients epilepsy started early in life. In addition to genetic factors (channelopathies, abnormal brain development), a so-called precipitating brain injury (stroke, TBI, SE, etc.) is identified as the cause of epilepsy in about one-third of epileptic patients. Mechanisms involved in development of acquired epilepsies are not yet fully understood, but blockade of epileptogenesis, which can fully prevent development of epilepsy in the future, attracts a lot of attention as a potential therapeutic target.

The immature brain is particularly susceptible to seizures. In contrast, many previous studies have concluded that the immature brain is more resistant to the development of symptomatic epilepsy than a mature one. Recent data, however, demonstrate that epileptogenesis can be induced even in very immature rodent brain by various insults, but the time-course of seizure development, seizure semiology, and both morphological and functional alterations are highly age-dependent. Already in P10–12 rats, epileptogenesis can be provoked in majority of animals. Our data with a model of early status epilepticus demonstrate that both severity of epilepsy and morphological impairment can progress with time, but also that severity of epilepsy does not simply correlates with the neuronal loss. In contrast to adult animals, functional as well as morphological alterations are much more delicate and period between the insult and occurrence of spontaneous seizures is longer, what makes a model of early status epilepticus more adequate to human situation. Future studies are however necessary to find out whether there are general model-independent mechanisms involved in epileptogenesis and whether suppression of these mechanisms does not have negative effects on functional recovery or adaptive changes involved in functional improvement.

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Symposium

Metabotropic glutamate receptors and depression

Peripheral metabotropic glutamate receptors: focus on the role of mGluR7 in brain-gut axis regulation

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Metabotropic glutamate receptors (mGluRs) and particularly mGluR7 are attractive targets for treatment of mood disorders, as supported by pharmacological and genetic studies. mGluRs also have various roles in the periphery including the gastrointestinal (GI) system; interestingly, mood disorders are frequently associated with GI dysfunction, an example of the bidirectional connection also known as the “brain-gut axis”. However, the contribution of mGluR7 to GI function remains unexplored. Therefore, we aimed to investigate a functional role of mGluR7 in the mouse colon by assessing agonist-induced alterations in colonic function. We found that mGluR7 activation increases faecal water content under acute stress as well as colonic secretory function *ex vivo*, thus confirming the functional relevance of colonic mGluR7. This suggests that targeting mGluR7 may not only be useful in the treatment of central components of stress disorders, but also stress-associated GI dysfunction such as diarrhoea or constipation.

Influencing metabotropic glutamate receptors in the immature brain

Pavel Mareš

Drugs affecting metabotropic glutamate receptors exhibit many actions: neuroprotective, antidepressant, anxiolytic and anticonvulsant. Primary concern of our laboratory is anticonvulsant activity of these drugs in developing brain, but positive side effects (from our point of view) would be of great value.

Antagonists of group I of metabotropic glutamate receptors exhibit marked anticonvulsant action in two models routinely used in our laboratory: pentetrazol-induced motor seizures and cortical epileptic afterdischarges. Antagonists of mGluR5 MPEP and MTEP were active in 12- and 18-day-old rats in both models, effects of an antagonist of mGluR1 AIDA reached the level of statistical significance only in pentetrazol model. None of these drugs compromised motor abilities of rat pups, spontaneous locomotion in open field was only marginally affected. Repeated exposure in the open field demonstrated that habituation was not
compromised. MPEP and AIDA exhibited anxiolytic-like effect in light-dark box, MTEP in elevated plus maze. AIDA did not affect learning of rat pups in homing test but MPEP worsened this ability. MPEP was also demonstrated not to induce neuronal death in contrast to antagonists of NMDA receptors. To conclude: antagonists of group I possess anticonvulsant and anxiolytic properties also in immature rats but their effects on learning and memory should be tested in detail.

Drugs specific for metabotropic glutamate receptors belonging to groups II and III did not exhibit anticonvulsant action in our models. On the contrary some indications of proconvulsant effects were observed.

Zinc and magnesium interaction with glutamate system in depression

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Zinc (Zn) and magnesium (Mg) are natural modulators of glutamate system. Antidepressant-like properties of Zn have been demonstrated in the forced swim and tail suspension tests and olfactory bulbectomy, chronic unpredictable and chronic mild stress models. Likewise, Mg was active the forced swim test. Moreover, these bio-metals enhance antidepressant activity of conventional antidepressants in the forced swim test (FST). Recent studies indicate the involvement of glutamate receptors in antidepressant-like activity of Zn and Mg in the FST in rodents. Thus, Zn and Mg activity in the FST was antagonized by N-methyl-D-aspartic acid (NMDA, agonist of the NMDA receptor) or NBQX (antagonist of AMPA receptor) pretreatment. On the other hand, deficiency of Zn or Mg ions has been related to depressive disorders. Several groups demonstrated that clinical depression might be accompanied with lower serum Zn and/or Mg concentrations.

Moreover, our recent data demonstrate reduced Mg (but not Zn) level in prefrontal cortex and hippocampus and reduced affinity of Zn and Mg to glutamate NMDA sites labeled with [3H]MK-801 in hippocampus of suicide victims compared to age-matched controls.

All the data indicate the involvement of Zn/Mg interaction with glutamate system in the antidepressant activity of these bio-metals and suggest such mechanism in human depression.

Antidepressant-like activity of metabotropic glutamate receptors

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Glutamate, the main excitatory neurotransmitter in the central nervous system, is strongly involved in pathology of depression. Glutamate acts through different type of receptors, divided into ionotropic and metabotropic glutamate receptors. Metabotropic glutamate receptors are more promising as therapeutic targets than ionotropic, as their activation modulate the activity of neurotransmitter.
Among all synthesized ligands so far, mGlu5 receptor antagonists (MPEP, MTEP) exerted greatest antidepressant-like activity in most animal tests and models of depression, such as Porsolt, tail suspension and olfactory bulbectomy. Similar results were obtained for mGlu2/3 receptor antagonist MGS0039. Among the third group of mGlu receptors only mGlu7 was shown to be involved in depression so far, as its positive modulator AMN082 exerts antidepressant-like activity; the efficacy of mGlu4 receptor ligands was not satisfactory so far, as nor its selective agonists LSP1-2111 or PHCCC, neither nonselective agonist ACPT-I exerted such an activity in animal models. The selective agonists of mGlu8 receptors are lacking at present.

The involvement of mGlu receptors in the mechanism of action of commonly used antidepressants and in the pathology of depression was also studied. Using Western blotting procedure we showed that the level of mGluR5 receptor protein was increased in CA1 and decreased in CA3 region of the hippocampus. Our results further indicate that mGluR5 can possibly be engaged in the mechanism of depression. In the olfactory bulbectomy model of depression the decreases of mGlu2/3 and mGlu7 receptors were observed and those effects were reversed by chronic amitriptyline treatment. Chronic antidepressant (citalopram) treatment caused also decreases of mGlu7, but not mGlu4 receptor level in the frontal cortex and hippocampus of rats, brain. The results support the idea about the involvement of mGlu7, but not mGlu4 receptors in depression.
Ischemic stroke – treatment for acute stage

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The incidence of ischemic stroke is about 200 per 1 million population, 70% are first ever and 30% are recurrent. Among all new cases about 30% will die during the year and 35% will be permanently disabled. There are four effective treatments for acute ischemic stroke: organized multidisciplinary care in stroke units (all strokes), thrombolysis, aspirin and decompressive hemicraniectomy. Despite of many experimental and clinical studies there is no effective neuroprotective (against biochemical and inflammatory reaction) treatment. Intravenous thrombolysis with recombinant tissue plasminogen activator (rtPA) with 4.5 hours time window is recently most effective medical therapies for acute ischemic stroke with acceptable safety profile. The most feared complication of rtPA therapy is symptomatic intracerebral hemorrhage. Its use is limited by strict treatment criteria. These include narrow time window, age within 80 years and also by lack of appropriate pre- and in-hospital medical care and low awareness of patients and their families. The benefits of intra-arterial over intravenous thrombolysis are intuitive but have yet to be proven. Ongoing trials are investigating the benefits of novel thrombolytic agents (such as tenecteplase and desmoteplase) or the delivery of transcranial sonolysis in conjunction with systemic microspheres, which have been shown to improve delivery of rtPA and increase clot lysis. Large vessel occlusion is less likely to be recanalized by conventional rtPA therapy. Mechanical thrombectomy devices are currently evaluating in randomized trials. Decompressive craniotomy can be life-saving intervention for people with raised intracranial pressure occurring as a result of infarct-related cerebral oedema.

Pharmacological treatment of neurodegenerative diseases

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Neurodegenerative diseases are an increasing clinical problem. Pharmacotherapy offers very little or nothing to the increasing number of patients deteriorating in their cognitive and motor skills. Because of an obscure pathogenesis of the sporadic neurodegenerative diseases, many investigations are aimed at the role of.
various factors that may aggravate or change the course of the disease. The local and systemic inflammations evoked by neuronal death are obviously involved in the pathological process of neurodegeneration. Local inflammation is a source of potentially neurotoxic compounds, such as: free radicals, glutamate, complement, metalloproteinases, proinflammatory cytokines, that secreted in excess may aggravate the injury. On the other hand, the processes of regeneration and recovery take place during local inflammation, and are stimulated by trophic factor production. Systemic inflammation consists of peripheral activation of immune system and leads to autoimmune reaction against brain antigens. This may begin an autoimmune disease or a process of benign autoimmunity, eventually neuroprotective. There are strong suggestions that lack of the control of inflammatory reaction may lead to neurodegeneration. Although experimental data show that inhibition of local inflammation or enhancing of autoimmune reaction may be beneficial to neurons and may diminish the damage caused by toxins, mechanical injury and ischemia, clinical trials are not so successful till now.

Recent developments in multiple sclerosis treatment
Dagmara Mirowska-Guzel

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system. It is traditionally considered to be of autoimmune origin. There is growing evidence that inflammatory pathology dominates in early stage of the disease while neurodegenerative processes characterize the latter, progressive course of MS. As the etiology of the disease is still unknown, MS is not completely curable. The initial therapeutic strategies were directed at immune modulation and inflammation control, however the efficacy of current therapies such as interferones, glatiramer acetate, mitoxantrone are moderate. Lately, natalizumab joined the group of therapeutics used in relapsing MS, however serious adverse effect, e.g. progressive multifocal leucoencephalopathy limits its use. Although currently used drugs diminish relapse rate, their impact on disability progression is still uncertain. There is a need to improve treatment efficacy and make it more comfortable for patients. Some new, orally administered drugs such as: fingolimod, cladribine, fumaric acid, laquinimod, teriflunomid are investigated. Another promising groups are monoclonal antibodies designed to reduce inflammatory process in relapsing MS and neuroprotective agents for progressive disease. Also new forms of treatment such as induction or escalating therapy are proposed. Although newer therapies for relapsing MS are intended to be more effective and easier to administer they may also carry greater, still not defined risk. Effective treatments for progressive and rapidly evolving MS is still not available.
The search for new opioid peptides as analgesics has been mainly directed to develop analogs with higher biological potency, resistance against enzymatic degradation and receptor selectivity. Evidences suggest an important role of δ-opioid receptor in antinociception. Furthermore, δ-opioid agonists are very promising tools in pain therapy due to their lower addiction potential, lack of respiratory depression and tolerance.

The aim of the study was to evaluate whether the newly synthesized peptides, the analogs of deltorphins such as DEL-6 and DK-4, highly potent δ-opioid receptor (mouse vas deferens assay) agonists and enkephalin analog cUENK6, with very high μ-receptors (guinea pig ileum assay) agonistic potency, induce an antinociceptive effect in the hot-plate, and tail-immersion tests after intracerebroventricular (icv) administration in rats. The effects of peptides were compared to morphine antinociception. Our study indicated that cUENK6 at the doses of 0.25 nmol produced equal, but at the dose of 0.5 nmol stronger than morphine (13 nmol), antinociceptive effect in both tests. DEL-6 and DK-4 at the dose of 20 nmol produced antinociceptive effect comparable with morphine (13 nmol, icv) in the tail-immersion test. Furthermore, rats with developed tolerance to morphine indicated cross-tolerance to antinociceptive effects of cUENK6 but not to DEL-6. More detailed study indicated that δ-opioid receptor antagonist – naltrindole very strongly and, to the lower extent, μ-opioid receptor antagonist – β-funaltrexamine, inhibited antinociceptive effect of cUENK6 and DEL-6 or DK-4 in the tail-immersion test. Nor-binaltorphimine, a κ-opioid receptor antagonist, did not influence these effect.

These data suggest 1) a dominant role of δ- but to a lesser extent μ-opioid receptors in antinociceptive effect of these peptides and 2) existence of functional interactions between μ- and δ-receptors in the modulations of antinociception.
Opioids and neuropathic pain

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The therapy of neuropathic pain causes some problems for today's medicine. Classical analgetics, e.g. opioid receptor agonists, possess low activity, whereas atypical agents, e.g. antidepressants, anticonvulsants, and NMDA receptor antagonists have good therapeutic efficacy but also exert many adverse effects.

Insensitivity of neuropathic pain to opioid analgesics is difficult to explain. Some investigators suggest that this phenomenon can be connected with hyperglycemia accompanying diabetes. The loss of opioid receptors expression on C-fiber afferents, the activation of NMDA receptors, the increase of synthesis of NO, the increase in the levels of cholecystokinin and accumulation of morfine-3-glucuronide may also lead to reduction of sensitivity to morphine in neuropathic pain states.

The experimental model of neuropathic pain caused by the administration of streptozotocin (known as a painful diabetic neuropathy model) and the pain model caused by vincristine administration (known as a vincristin-induced toxic neuropathy model) were developed and used in experiments performed to investigate the mechanisms of neuropathic pain.

The aim of the conducted studies was to evaluate the influence of commonly used opioid analgesics on hyperalgesia induced by streptozotocin or vincristine. The influence of magnesium on investigated drugs activity was also studied.

The studies were performed on male Wistar rats. The changes in nociceptive thresholds were determined by using mechanical stimuli (the modification of the classic paw withdrawal test described by Randall and Selitto).

Diabetes was induced by intramuscular administration of streptozotocin at a dose of 40 mg/kg of body weight, as described by Nakhoda and Wong. The streptozotocin-induced hyperglycemia was accompanied by development of irreversible hyperalgesia.

Toxic neuropathy was induced by daily administration of vincristine at a dose of 70 mcg/kg into the tail vein. During this time hyperalgesia was observed. After discontinuation of vincristine administration, the nociceptive threshold to mechanical stimuli gradually increased to baseline value.

The results of the undertaken study showed, that fentanyl and buprenorphine only slightly alleviated, while morphine did not modify the streptozotocin hyperalgesia. All investigated opioids did not alter the vincristine hyperalgesia. Pre-treatment with magnesium markedly enhanced and prolonged the analgesic activity of morphine, fentanyl and buprenorphine in toxic neuropathy model. In the diabetic neuropathy model, a pre-treatment with magnesium also significantly enhanced the opioids' effects. However, in comparison with vincristine model, this effect was less pronounced. Assuming that magnesium will also not increase the opioid respiratory depression, something remains to be demonstrated, potentiation of opioid analgesia in neuropathic pain can be of great clinical importance.
Opioid-substance P chimeric peptides

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Our group proposed to develop new chimeric analgesics in which opioid pharmacophores are covalently hybridized with other types of pharmacophores that positively modulate effects of the opioid part. Synergistic enhancement of opioid analgesia and/or decrease of unwanted side-effects should result from such hybridization.

It is generally accepted, that opioids and substance P are classified as functional antagonists. However, their spectrum of interactions is much more complicated. Series of new opioid agonist-substance P antagonists and opioid agonist-substance P agonist conjugates have been synthesized and tested. Hybridization of opioids with tachykinin receptor ligands resulted with new properties that are dependent on their agonist or antagonist nature. In general, hybridization of opioid agonists with tachykinin antagonists resulted in strong analgesia evidencing synergistic interaction between opioids and tachykinin elements. In contrary, the substance P agonists may partially reduce opioid analgesic potency of chimeric compounds, but its presence strongly reduces side effects of opioids. In conclusions, both types of hybridized opioid-substance P ligands are interesting but with different prospective clinical applications.

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Analgesia in intensive care unit

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Therapy of Pain in Intensive Care Unit is subordinated to many elements of process of care; like diagnostic procedures or organ support.

Due to variety of patients and clinical problems clinical search of optimal analgesic is focused on pharmaceutical agents with possible minimal potential to cause adverse reaction to treatment.

Standard pain therapy has limited application in patients with multi organ failure.

Patients in critical care unit are sometimes very agitated. To make them tolerate mechanical ventilation they require combined drug treatment. The desired effect is: calming down, sedation and analgesia. This can be rarely achieved with single drug therapy. Combined therapy is very popular in ICU. Not only analgesics are used.

Of particular value is synergism of drugs since it helps to reduce posttraumatic stress reaction. This is very useful when patients are weaned from mechanical ventilation once they receive infusion of analgesics.

The best choice of drug and its dosage is another therapeutic problem. It is also challenge for doctors: how to provide effective analgesia and adjust treatment individually to patients with multi organ failure.
heart failure and circulatory insufficiency, changed liver metabolism, renal insufficiency and renal replacement therapy. Drug interactions and side effects may be the reason to abandon certain treatment options.

Some routes of application will never be used: oral rectal or percutaneous application may have unpredictable pharmacokinetics of given medication. Intravenous analgesia is most frequently used in ICU because it assures the most reliable steady state of infused analgesics.

Very popular and effective pain treatment technique: Patient Controlled Analgesia PCA has very limited application since many patients in Intensive Care are unconscious and can not use the pomp to titrate analgesia according to their individual needs.

Alternative way of drug infusion comprise epidural and spinal space or regional nerve blocks.
Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder beyond Alzheimer’s disease, affecting approximately 1% of people over the age of 65. The major pathological hallmarks of PD are significant loss of nigrostriatal dopaminergic (DA) neurons and the presence of intraneuronal protein inclusions termed Lewy bodies (LB). Alpha-Synuclein (ASN) is a major component of LB and play significant role in pathogenesis of PD and is implicated in the other neurodegenerative disorders. However, the molecular mechanism of ASN mediated neuronal cell death is unclear. The current data demonstrate the presence of ASN and its aggregated forms in the extracellular fluid both in vivo and in vitro. Our study indicated that ASN is liberated from synaptoneurosomes into extracellular space during oxidative/nitrosative stress. We observed that exogenous ASN at monomeric/oligomeric form disturbs dopamine transporter (DAT) and voltage-dependent calcium channels (VDCC) function in the brain. ASN-evoked disregulation of Ca^{2+} homeostasis leads to activation of neuronal nitric oxide synthase (nNOS). Our data showed NO-dependent modification of several proteins involved in decision of cell life/death including caspase-3 and poly(ADP-ribose) polymerase 1 (PARP-1). The studies on neuronal cells in culture presented ASN/NO dependent mitochondria failure and indicated that NO pool liberated by ASN activates caspase-3 that leads to PARP-1 cleavage. Inhibitor of NOS (NNLA), caspase-3 (Z-DEVD-FMK) and a mitochondrial permeability transition pore blocker, cyclosporine A had protective effect against ASN evoked cell death. Our results indicate that ASN leads to NO mediated mitochondria dysfunction and caspase-dependent programmed cell death. Thus, extracellularly acting ASN may be an important factor in degenerative changes and it potentially provides therapeutic targets for retarding the progression of the neurodegeneration.

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Role of mitochondria in age-related neurodegenerative diseases

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Analysis of the protein profile of organisms and its age-dependent variation is a promising approach to unravel mechanisms involved in ageing and age-related diseases. Our studies focus on the mammalian mitochondrial membrane proteome, especially of the inner mitochondrial membrane with the respiratory chain complexes and other proteins possibly involved in life span control, ageing and neurodegenerative disorders. Variations of the mitochondrial proteome during ageing, with the emphasis on the abundance, composition, structure, interactions, post-translational modifications and activity of membrane proteins, are examined in various rat tissues by native polyacrylamide gel electrophoresis techniques in combination with mass spectrometry.

In rat brain, age-modulated differences in the abundance of various non-mitochondrial and mitochondrial proteins are detected. The age-related alterations in the abundance and oligomerisation of the MFoF1 ATP synthase observed by us in rat cortex might be one clue for understanding the link between respiration and longevity. Also, the abundance of OxPhos (Oxidative Phosphorylation) supercomplexes, the natural assemblies of the respiratory complexes I, III, and IV into supramolecular stoichiometric entities such as I1III2IV0-4, differs in young and aged cortex tissue [Seelert et al., Biochim Biophys Acta 1787, 2009]. The mitochondrial proteome profiles of the hippocampus and striatum are compared with that of the cortex, reflecting differential brain ageing. Investigation of the influence of life-long and short-term calorie restriction (CR) on the mitochondrial liver protein profile will be discussed in relation to the observed anti-ageing and pro-ageing effects of CR. The combination of the blue-native (BN) gel system with fluorescence difference gel electrophoresis (DIGE) favours an efficient and highly sensitive quantitative analysis. Alterations in the level of reactive oxygen species (ROS), in anti-oxidative status and lipid composition are considered. The data discussed and methodologies described are also of high relevance for elucidating the molecular basis of age-related diseases such as Alzheimer’s and Parkinson’s Disease. The restorative effects of 9-methyl-β-carboline in an rat model of Parkinson’s disease might be associated with the nearly 3-fold increase in the activity of the OxPhos supercomplex I1III2IV2 [Wernicke et al., Pharmacol Rep, 2010]. Applying neutron diffraction, we could demonstrate that AD amyloid-β peptides insert in lipid membranes and perturb their structure and dynamics. Cholesterol hinders the intercalation of monomeric Aβ completely [Seelert et al., Biochim Biophys Acta 1787, 2009].
Inhibition of proteasome function in rat nigrostriatal dopaminergic neurons as an animal model of Parkinson’s disease for studying neuroprotective compounds

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Impairment of the ubiquitin-proteasome system was suggested as a potential etiopathogenic factor of Parkinson’s disease (PD). The experimental modeling of proteasome function in rat nigrostriatal dopaminergic neurons was based mainly on intranigral administration of selective proteasome inhibitors, despite the fact that intrastriatal injections were also applied. The aim of the present study was to ascertain whether inhibition of the proteasome function in nigrostriatal dopaminergic neurons is a good animal model of PD for studying the neuroprotective properties of different compounds. We examined the effects of 1,2,3,4-tetrahydroisoquinoline (TIQ), an endogenous amine occurring in brain, cinnarizine, a calcium channel antagonist, and celastrol, a potent antioxidant, on dopamine (DA) metabolism. All those compounds were administered systemically to rats injected unilaterally with a single dose of the selective proteasome inhibitor lactacystin (1 µg/2 µl) into the SNc. The experiment was conducted on a few lactacystin-treated groups which received ip TIQ (50 mg/kg; 7 days), cinnarizine (10 and 30 mg/kg; 7 days) or celastrol (3 mg/kg; 4 days). Control rats were injected intranigral with a solvent and ip with the examined compounds. On day 8 after lactacystin, the rats were killed by decapitation. The levels of DA and its metabolites were determined in striatal homogenates using an HPLC method. At that time, lactacystin-treated rats showed a significant decrease in the levels of DA and its metabolites in the ipsilateral striatum. As to DA catabolism, lactacystin accelerated the total (HVA/DA) and MAO-dependent oxidative (DOPAC/DA) DA catabolism in that striatum. Subchronic TIQ and cinnarizine administration prevented the loss of striatal DA and attenuated the lactacystin-enhanced DA catabolism in the ipsilateral striatum, whereas celastrol evoked no such effects. The obtained results suggest that TIQ and cinnarizine can act as neuroprotective agents. Celastrol is devoid of such properties. Putative mechanisms of the neuroprotective activity of TIQ and cinnarizine are discussed in the context of PD.

Symptomatic and neuroprotective properties of adenosine A2A receptor antagonists in animal models of parkinsonism

Jadwiga Wardas

Recently, adenosine A2A receptor (A2AR) antagonists stand as an attractive non-dopaminergic therapeutic target in Parkinson’s disease (PD), based on preclinical pharmacological and molecular data, and, in part upon a unique CNS distribution of this receptor. These compounds both alone and as adjunctive therapy in combination with low doses of L-DOPA relieve motor deficits in animal models of PD, which was suggested to result from the well-documented antagonistic interaction between A2AR and dopamine D2 receptors in striatopallidal neurons. Converging epidemiological and experimental evidence has raised the
possibility that $\alpha_2A$R antagonists may also represent a novel neuroprotective strategy for PD. The neuroprotective effects of $\alpha_2A$R antagonists (KW6002, SCH58261, MSX-3) were studied in acute and chronic animal models of PD (chronic MPP$^+$, MPTP and 6-OHDA). These compounds partially reversed the toxin-induced loss of dopamine (DA) and its metabolites (DOPAC, HVA) in the striatum (CP), and the decrease in the number and density of TH-ir neurons in the substantia nigra pars compacta (SNc), estimated stereologically. Moreover, MPTP-induced loss of DA neurons in SNc and CP, associated with the increased astroglial (GFAP) and microglial (CD11b) immunoreactivity, was also attenuated by $\alpha_2A$R antagonists. Since $\alpha_2A$R antagonists control the action of growth factors which play an important role in the neuroprotective effects on DA neurons, their action on BDNF mRNA was also examined in different brain structures. In conclusion, the results provide evidence for neuroprotective effects of $\alpha_2A$R antagonists in animal models of PD. Together with the symptomatic antiparkinsonian potential of several $\alpha_2A$R antagonists being pursued in clinical trials, these compounds may afford dual benefits.

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