Doi: 10.1038/aps.2013.107

PL-1

Brain-derived neurotrophic factor (BDNF) as an endogenous antioxidant in animal models of disease

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BDNF is now known to possess nontrophic actions, one of which is amelioration of brain stem cardiovascular dysregulation via an antioxidant effects in rostral ventrolateral medulla (RVLM), a key nucleus of the baroreflex loop. In a temporal lobe status epilepticus (TLSE) model, hypotension preceded by reduced baroreflex-mediated sympathetic vasomotor tone accompanies sustained hippocampal seizure; with concurrent increases in superoxide, phosphorylated $p47^{phox}$ NADPH oxidase subunit, BDNF mRNA or protein, tropomyosin receptor kinase B (TrkB) or angiotensin receptor subtype 1 (AT1R) in RVLM. Whereas superoxide dismutase mimetic (tempol), NADPH oxidase inhibitor (apocynin) or AT1R antagonist (losartan) blunts the elevated superoxide or phosphorylated $p47^{phox}$ subunit, hypotension and the reduced baroreflex, a recombinant TrkB-Fc fusion protein or an antisense bdnf oligonucleotide potentiates all those events. In a neurogenic hypertension model, angiotensin II (Ang II) upregulates BDNF mRNA and protein, induces phosphorylation of cAMP response element binding protein (CREB) or $p47^{phox}$ subunit, suppresses mitochondrial electron coupling capacity, and increases mitochondrial uncoupling protein 2 (UCP2) in RVLM. Tempol, apocynin or antisense CREB oligonucleotide blunts the Ang II-induced BDNF upregulation; and gene knockdown of BDNF or depletion of TrkB attenuates UCP2 upregulation or potentiates Ang II–induced superoxide and pressor response. We conclude that BDNF acts as an endogenous antioxidant in RVLM in TLSE and neurogenic hypertension.

Keywords: BDNF; antioxidant actions; neurogenic hypertension

PL-2

The stability of NR2B in the nucleus accumbens controls behavioral and synaptic adaptations to chronic stress and amphetamine

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Aim: The nucleus accumbens (NAc) is closely correlated with psychiatric disorder. It has been demonstrated that the glutamatergic system in NAc plays an important role in the reward pathway. We therefore tested whether *N*-methyl-*D*-aspartate receptors and the synaptic plasticity in the NAc are regulated by chronic stress and amphetamine. **Methods:** Social defeat stress, social interaction, sucrose preference, golgi staining, western blotting and electrophysiological methods (field potentials recording) were used. **Results:** We found that chronic stress and chronic exposure to the psychostimulant amphetamine induced selective downregulation of *N*-methyl-*D*-aspartate receptor NR2B subunits in the confined surface membrane pool of NAc neurons. Remarkably, the loss of synaptic NR2B was a long-lived event and further translated to the significant modulation of synaptic plasticity in the form of long-term depression. We further observed that the stress-induced changes were restored by fluoxetine and that resilient mice—those resistant to chronic stress — showed patterns of molecular regulation in the NAc that overlapped dramatically with those seen with fluoxetine treatment. Furthermore, incubation of ubiquitin-proteasome system inhibitor MG-132 reversed the reduction of NR2B in homogenate from amphetamine-treated rats. Behaviorally, restoration of NR2B loss prevented the behavioral sensitization of mice to chronic stress and amphetamine. **Conclusion:** Our results identify NR2B in the NAc as a key regulator in the modulation of persistent psychomotor plasticity in response to chronic stress and amphetamine.

Keywords: nucleus accumbens; NR2B; synaptic plasticity; chronic stress; amphetamine

Acknowledgements: This work was supported by grant from the 973 Programme of China (No 2013CB531303), the Key Project of National Nature Science Foundation of China (No 30930104).

PL-3

GABA receptor allosterism

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The two major classes of γ-aminobutyric acid (GABA) receptors are designated $GABA_A$ and $GABA_B$. Whereas the $GABA_A$ receptor is a pentameric, ligand-gated ion channel, the $GABA_B$ site is a heterodimeric, G protein-coupled receptor (GPCR). Both were initially characterized using orthosteric agents, such as muscimol and bicuculline for $GABA_A$ and baclofen for $GABA_B$. After the discovery that benzodiazepines interact allosterically with the $GABA_A$ site, attention turned to determining whether other agents act in a similar manner. Among those identified were ethanol, barbiturates, and picrotoxin. Given earlier work on allosterism and the pentameric, ligand-gated cholinergic nicotinic receptor, it was not surprising that the $GABA_A$ receptor can be pharmacologically manipulated in this way. In contrast, research on the $GABA_B$ receptor focused on developing orthosteric agents, as this is the conventional approach for pharmacologically modifying GPCRs. However, because of the widespread distribution of $GABA_B$ receptors, and the lack of distinct receptor subtypes, the side effects associated with orthosteric agonists and antagonists limit their therapeutic potential. Prospects for developing new $GABA_B$ therapeutics improved considerably with the discovery that this site is also subject to allosteric modulation. Indeed, $GABA_B$ receptor subtype-selective allosteric agonists have now been synthesized that display anxiolytic activity without causing the sedation, muscle relaxation and cognitive dysfunction typically associated with orthosteric compounds. In light of earlier work suggesting that orthosteric $GABA_B$ receptor agents display analgesic, antidepressant, and anxiolytic properties, and can enhance cognition and foster neuroregeneration, the development of allosteric modulators for this site could lead to the identification of novel drugs for treating a host of central nervous system disorders.

PL-4

Regulation of cell functions by Ca^{2+}: from basic principles to therapeutic targets

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Intracellular Ca^{2+} signaling regulates numerous cell functions including contraction, secretion, and immune responses. There are many potential molecular targets for drug development in conjunction with the cell functions regulated by Ca^{2+} signals. For example, pharmacological interference with the Ca^{2+} signaling in the heart and blood vessels by calcium antagonists has been utilized for the treatment of hypertension and other cardiovascular diseases. Based on our initial study on the basic principles of Ca^{2+} signaling, we have been searching for new cell functions that are regulated by Ca^{2+} signals in the brain. We found that nitric oxide (NO) induces release of Ca^{2+} from the intracellular Ca^{2+} store through *S*-nitrosylation of the ryanodine receptor Ca^{2+} release channel in central neurons. Our results suggest that the NO-induced Ca^{2+} release is one of the causal mechanisms of NO-dependent neuronal cell death and can be a therapeutic target in certain forms of ischemic brain injury. In response to traumatic brain injury, astrocytes generate Ca^{2+} signals. We found that Ca^{2+}-dependent N-cadherin upregulation in astrocytes around the injury site is required for astrogliosis and neuroprotection. These new results highlight the pathophysiological significance of Ca^{2+} signaling in the brain, and the molecules involved the signaling pathways may serve as potential therapeutic targets.

PL-5

Human ABC transporters and pharmacogenomics

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ATP-binding cassette (ABC) transporters constitute a superfamily of membrane proteins. The ABC proteins protect cells from a wide range of toxic compounds, and regulate basic biologic processes of essential organs. Consequently, mutations affecting ABC-transporters have been found to be the underlying causes for a large number of human inherited diseases including cystic fibrosis. Currently, 49 ABC transporter genes were identified in human genome and classified into 7 subfamilies according to their sequence homologies. The multi-drug resistance 1 (MDR1) and multidrug resistance proteins (MRPs) genes were originally identified as a gene that confers multi-drug resistance to cancer cells. They have a physiological role of protecting cells and organisms from various toxic substances including commonly prescribed medications. Thus, identifying the individual genetic variations of these transporters carries significant meaning in pharmacotherapy of post-genomic era, since such genetic variants are likely to be an important source for the inter-individual variability in toxicity and response of many drugs. Our results of genetic association studies revealed that genetic variations in the ABC transporters are associated with drug response and toxicity of antidepressants, antiepileptics and cardiovascular agents. Further identification of the functional genetic variations in ABC transporters and other major drug-response genes using the state-of-the-

art technologies will improve the predictive value of genetic tests for tailored drug therapy.

PL-6

Current trends in the study of maps in view of their clinical uses

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Medicinal plants are a source of raw materials for both traditional and modern medicine. Some 80% of the inhabitants of the World relies chiefly on traditional medicines (Akerele, 1975). MAPs are increasingly employed as home remedies, OTC-drugs and/or ingredients for the pharmaceutical industry.

The demand to prove the efficacy and safety of MAPs has already gained ground with MAPs. The ultimate aim would be to use MAPs with well-defined and constant composition.

Clinical tests, aimed at single chemicals are not really adequate for herbs, since their effects are caused by the interaction several active principles.

MAPs are sourced from both wild-crafting and cultivation. Quality is primarily determined by the genotype, the environment and the conditions during the life cycle of the plant. Processing to preserve/isolate their active principles is an additional precondition for the safety and efficacy in use. National/international regulatory authorities have elaborated guidelines (GACP, GMP, and GLP) to be included in quality assurance systems. The entire production system should adhere to these practices and this fact should be continuously documented, certified.

There is also a need to ensure the quality of medicinal plant products by using modern sample preparation and control techniques as well as suitable standards.

Relevant information, research findings (on the safety/efficacy, the use in local health systems, clinical trials) should be shared more effectively. Harmonization is needed to secure the sustainable production and safe use of this valuable natural resource.

PL-7

Structure-function relationships of human UDP-glucuronosyl-transferases: Application of molecular, kinetic and computational modelling approaches

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The UDP-glucuronosyltransferases (UGTs) comprise a family of enzymes that are important for the metabolism of drugs, non-drug xenobiotics, and endogenous compounds. Individual UGT enzymes exhibit distinct, but overlapping, substrate selectivities. Although most UGTs have the capacity to metabolise small planar phenols and aliphatic alcohols, increasing complexity of the substrate confers selectivity due to steric, hydrophobic and polar interactions. Structure-function relationships, which have been inferred largely from chimeragenesis and site-directed mutagenesis, indicate that a near conserved N-terminal domain histidine plays a pivotal role as the catalytic base in the metabolism of phenols and aliphatic alcohols. However, substitution of this histidine with proline provides the capacity for UGT1A family enzymes to metabolise tertiary amine substrates. The complex kinetic behaviour observed for many of the human UGTs arises from homodimerisation. Notably, kinetic data for the enzyme UGT2B7 are consistent with the existence of two catalytic sites within the active site, along with multiple effector sites. Understanding the substrate profile of the human UGTs with respect to both drugs and endogenous compounds has further provided important insights into the modulation of enzyme activity in vitro by membrane long-chain unsaturated fatty acids. In turn, this has allowed the development of experimental paradigms that accurately drug kinetic parameters in vivo.

Keywords: drug metabolism, glucuronidation, UDP-glucuronosyl-transferase, enzyme structure-function, kinetics

PL-8

Guanidines: Pharmacology from molecule to primate

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Guanidine and its derivatives have drawn the attention as therapeutic and potential therapeutic agents some 40 years ago in view of the bioactivities of hydroxy urea and guanidine in medicinal fields amongst others in cancer and virology. Guanidine with its unique basic structure lends itself to opportunities of development of various derivatives. Guanidine-type structures such as the cyanoguanidines have found clinical application drugs in the treatment of hypertension such as the potassium channel opener pinacidil and histamine-II-receptor antagonists (e.g. cimetidine). During the last decade several known and novel guanidine derivatives have been researched for various new medicinal applications in view of their interactions with enzyme systems such as xanthine oxidase and nitric oxide synthase. These properties revealed their potential to afford neuroprotection in the advent of brain injury.

During recent years we have investigated guanidine derivatives in the fields of HIV/AIDS, α2 adrenoreceptors, electron acceptors at the xanthine oxidase enzyme and ischaemic heart disease. These investigations revealed remarkable protective properties of a novel guanidine (N-(3,4,-dimethoxy-2-chlorobenzylideneamino)-guanidine: ME10092) on myocardial ischaemia and reperfusion in rats against induced myocardial necrosis and life-threatening arrthythmias. Subsequently, studies were conducted with this N-hydroxyguanidine (ME10092) on the Papio ursinus baboon investigation its effect on cerebral perfusion together with monitoring of the cardiovascular parameters. These *in vivo* non-human primate investigations indicated no significant cerebral perfusion effects using the split-dose single photon emission computed tomography (SPECT) at the dose and time-schedules applied in these studies. However, negative chronotropic effects and changes in blood pressure were induced by ME10092. These effects of ME10092 in the baboon strengthen the findings found in the rodent studies.

In conclusion guanidine-like compounds have over four decades exhibited pharmacological actions in diverse medicinal fields with a variety of therapeutic applications. The guanidine structure have proved to be an important scaffold in the design and development of clinically important drugs and novel compounds could in the future add to the therapeutic range already in existence.

PL-9

Glucocorticoid insensitivity in chronic inflammatory diseases

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Glucocorticoid (GC) insensitivity is a major limitation in the treatment of certain types of cancer and chronic inflammation, including asthma and chronic obstructive pulmonary disease (COPD). Glucocorticoid insensitivity has been extensively investigated in inflammatory cell types. In these cell types there has been a near-exclusive focus on transrepression mechanisms as the source of the resistance, as transrepression effects of GC are held to explain the major anti-inflammatory effects of GC. The predominance of transrepression has been questioned by several findings including the demonstration that annexin-A1, a gene controlled by transactivation, has a key role in the impact of GCs in murine models of peritonitis and arthritis. Moreover, the range of cellular targets for beneficial actions of GCs is much wider than inflammatory cells. Airway structural cell types (smooth muscle, fibroblasts, epithelium) are important targets for GC action in in asthma and COPD. We have identified that degraded collagen, certain mitogens and cytokines as induce insensitivity to transactivation actions of GC in airway smooth muscle (ASM) and have recently discovered that the fibrogenic cytokine, Transforming Growth Factor-β (TGF-β) induces profound impairment of GC transactivation in airway epithelial cell lines and primary cultures as do interleukin-13 (IL-13), IL-4, and TNF-α, individually and in combination. Endogenous TGF-β contributes to GC insensitivity in respiratory syncytial virus (RSV). As viral infection accounts for the vast majority of GC-resistant exacerbations of severe asthma and COPD, these new findings identify strategies to target common pathways in GC resistance.

Keenan CR, Salem S, Fietz ER, Gualano RC, Stewart AG. Glucocorticoid-resistant asthma and novel anti-inflammatory drugs. Drug discovery today. 2012; 17 (17–18): 1031–8.

PL-10

Therapeutic targetting of endothelin and nitric oxide in hypertension and chronic kidney disease

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Chronic kidney disease (CKD) is a common and growing public health problem, strongly associated with high blood pressure (BP), and causing substantial cardiovascular mortality and morbidity. CKD is also associated with endothelial dysfunction and arterial stiffness, independent predictors of poor outcome. Proteinuria is an added risk factor linked to underlying renal pathology. A drug that reduces BP, proteinuria and arterial stiffness would be of significant therapeutic potential in CKD. Studies in animal models and patients show that nitric oxide bioavailability (NO) is reduced, and the vascular endothelin (ET)

system overactive, in CKD. Both systems, acting in opposition, may be useful therapeutic targets. Here, I will review the evidence that ET receptor antagonists (ETRAs; blocking ET actions) and phosphodiesterase type 5 (PDE5) inhibitors (prolonging NO-linked cGMP mediated effects) are anti-inflammatory and can reduce BP, arterial stiffness, and proteinuria in CKD. The focus is on key preclinical studies and clinical trials with ETRAs and the PDE5 inhibitor, sildenafil. Although there is less evidence for proteinuria reduction with PDE5 inhibitors, they do not cause fluid retention (unlike ETRAs) and have a good safety profile. Although this area looks extremely promising for clinical development, further studies are needed to confirm the benefits, followed by larger studies to examine whether the overall balance of efficacy and safety supports the use of these drugs in addition to standard treatment in patients with hypertensive CKD.