Journal of Biomedical Science (2007) 14:695–698
DOI 10.1007/s11373-007-9206-y

The Vignette for V14 N6 Issue

The vicissitudes of the pacemaker current IKdd of cardiac Purkinje fibers

The pacemaker potential in cardiac Purkinje fibers has been attributed to the decay of the potassium current IKdd [1]. An alternative proposal is that the hyperpolarization-activated current If underlies the pacemaker potential in all cardiac pacemakers [2]. The aim of this review [3] is to retrace the experimental development related to the pacemaker mechanism in Purkinje fibers with reference to findings about the pacemaker mechanism in the sinoatrial node. Major experimental data were attributed to K+ depletion in narrow extracellular spaces which would result in a time-dependent decay of the inward rectifier current IK1. In order to avoid such a postulated depletion, Ba2+ was used to block the decay of IK1. However, Ba2+ had also blocked IKdd (and not only IK1). In single Purkinje cells in the absence of Ba2+, IKdd was present in the pacemaker potential range and reversed at EK. In the presence of Ba2+, IKdd was blocked and If appeared at potentials negative to the pacemaker range [4]. The pacemaker potential behaves in a manner consistent with the underlying IKdd but not with If. It is concluded that the large boy of evidence supports the pacemaker role of IKdd (but not of If) in Purkinje fibers.

The dominant-negative action of a fusion protein containing the cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 in virus replication

The cytoplasmic domain of envelope (Env) transmembrane protein gp41 interacts with the MA protein of Gag during assembly/budding of HIV-1. Chan and Chen have previously reported that the cytoplasmic tail of gp41 (amino acid 706–856) fused with β-galactosidase (β-gal/706–856 fusion protein) down-regulates the expression of Gag, probably via an intracellular protein degradation pathway [5]. They [6] now show that this β-gal/706–856 fusion protein displays a dose-dependent dominant interference with virus infectivity. In the context of an HIV provirus, in addition to Gag, β-gal/706–856 also down-regulates the steady-state expression of Env protein, which leads to the interference of virus infectivity and replication. However, the overexpression of Env suppresses β-gal/706–856-mediated Gag down-regulation. Sucrose gradient ultracentrifugation and confocal microscopy show that Gag, Env, and β-gal/706–856 have stable interaction and can form a complex in the perinuclear region. Together with results from cytoplasmic tail mapping analyses, redirection of Gag from its cytoplasmic synthesis site to a perinuclear compartment is required for β-gal/706–856-mediated Gag down-regulation.

Suppression of hepatitis B viral gene expression by protein-tyrosine phosphatase PTPN3

Hepatitis B virus (HBV) is the major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The precise mechanism of HBV gene expression is not completely clear. The study by Hsu et al. in this issue [7] shows that a protein-tyrosine phosphatase PTPN3 can suppress the transcription of various mRNAs of HBV. This suppressive effect requires the phosphatase activity and the FERM (four protein-1, ezrin, radixin and moesin) domain of PTPN3 protein, but not the PDZ domain. This study thus identified a new cellular factor in regulating HBV gene expression. It will be interesting to identify the mechanism of PTPN3 involvement in HBV replication.

Transplantation of mesenchymal stem cells in myocardial infarction

The use of bone marrow-derived mesenchymal stem cells (MSCs) is an auspicious method which prevents deleterious remodeling and improves left ventricular (LV) function [8, 9]. However, there is no published data from a systematic study, which might indicate whether or not in vitro differentiation induction of MSCs has more beneficial

effects for the regeneration of the infarcted myocardium and improvement of LV function than undifferentiated MSCs. The goal of this study is to address this issue with an extensive investigation. Results demonstrated that cells without differentiation induction were as effective as cells with prior differentiation induction. In conclusion, directing MSCs toward cardiac cells before transplantation is not needed. Peri-infarct injection of marrow-derived MSCs can effectively regenerate infarcted myocardium and improve cardiac function [10].

Nitric oxide and blood pressure

The tail-cuff method for measuring systolic blood pressure (SBP) in rats is very popular. However, the accuracy of measurement of SBP by tail-cuff method was still doubted [11]. The present study was to clarify and explain the difference of SBP value obtained between the inflation (Inf) and deflation (Def) by tail-cuff. Results obtained from simultaneous measurement of SBP by tail-cuff method and carotid cannulation revealed that the Inf value was the most accurate estimation of intravascular SBP. This study suggested that nitric oxide accumulation due to flow deprivation was the main cause of SBP underestimation by Def values. Thus, the Inf value should be taken as representative of SBP, since, depending on the duration of suprasystolic compression, the Def value may underestimate SBP [12].

***Knock down* of gfp and no tail expression zebrafish embryo by in viro-transcribed short hairpin RNA with T7 plasmid**

The short hairpin RNA (shRNA) expression system containing T7 RNA polymerase and CMV promoter was developed to knock down fluorescent protein and no tail (ntl) mRNA in zebrafish embryo [13]. When pT7shGFP was injected in the transgenic embryo stably expressing T7RP, gfp showed a decrease in expression by 68%, as analyzed by real-time PCR. Meanwhile, the injection of SiRNA resulted in the decrease of expression level of 40% as compared with that of control. The injection of pT7shNTL vector in zebrafish embryo led to partial absence of ntl transcripts in 30% of injected embryos. Therefore, the T7 transcription system of shRNA expression could be used for RNAi studies in zebrafish embryo.

Differences in osteoblast miRNA induced by cell binding domain of collagen and silicate-based synthetic bone

MicroRNA expression was used to study the effects of pewoGlas (PG), silicated-based materials and P-15, an analog of cell binding domain of collagen on promoting bone formation by altering osteoblast activity. Three miRNas (mir-30b, mir-373, and mir-92) were up-regulated whereas eight miRNAs (mir-337, mir-377, mir-25, mir-2006, mir-129, mir-373, mir-133b, mir-489) were down-regulated [14]. PG and P-15 enhance the transcription of several mirRNAs of osteogenetic genes, but P-15 acts on homeobox genes.

siRNA targeting midkine inhibits gastric cancer cell growth and induces apoptosis through caspase-3, -8, -9 activation and mitochondrial depolarization

Midkine (MK), a heparin-binding protein is up-regulated in various malignant tumors. By using siRNA targeting MK in human gastric cancer cell lines, BGC823 and SGC701, the growth of both cell lines were significantly inhibited by knock-down of MK gene [15]. The loss of mitochondrial membrane potential, release of caspase-3, -8, -9 occurred by inhibiting MK gene. The results indicated that MK siRNA can inhibit gastric cancer cell growth and induce apoptosis. Therefore, MK siRNA could be a potential therapeutic agent for the treatment of gastric cancer.

Aberrant expression and distribution of the OCT-4 transcription factor in seminomas

The OCT-4 protein is a nuclear transcription factor belonging to class V of Pit-Oct-Unc (POU) family [16]. Up-regulation of OCT-4 gene in testicular seminomas and embryonal carcinomas [17] was reported. By using immunohistochemical staining and Western blotting, Cheng et al. [18] demonstrated that OCT-4 was aberrantly localized in the cytoplasm and nuclei of cells

in seminoma tissues. This result was further confirmed using immunocytochemical staining of NCCIT (seminoma-embryonal carcinoma) and NT2 (embryonal carcinoma) cells. In NCCIT, NT2 cells and seminoma tissues, OCT-4 mRNA was highly expressed. The aberrant expression and distribution of OCT-4 in seminomas may contribute to cell transformation between germ line stem cells and testicular germ cell tumors.

Regulation of extracellular glutamate levels in the long-term anoxic turtle striatum: coordinated activity of glutamate transporters, adenosine, K + ATP channels and GABA

In contrast to the anoxic mammalian brain which experiences an uncontrolled release of glutamate accompanied with excitotoxic cell death, the turtle brain shows no increase in extracellular glutamate levels over many hours of anoxia [19]. In the anoxic turtle brain, there is a decrease in glutamate release combined with continued glutamate reuptake by glutamate transporters [20]. Thompson et al [21] reported in this issue that alterations in glutamate release during early anoxia are modulated by adenosine receptors and K (ATP) channels but not by GABAA receptors. In long-term anoxia, there was a more substantial decrease in extracellular glutamate level that was regulated by adenosine and GABAA receptors but not by K (ATP) channels. The respective contributions of adenosine, GABAA receptors and K (ATP) channels in glutamate regulation differ, therefore, between early and long-term anoxia. Since the maintenance of glutamate transport is an energetically expensive process, it is likely that decreased glutamate release in combination with glutamate reuptake comprises a critical neuronal survival mechanism. These observations strongly suggest that glutaminergic blockade may be an inappropriate therapeutic strategy for ischemic stroke and that the maintenance of low-level glutaminergic function is a key contributor to anoxic brain survival.

Neuroprotection and free radical scavenging effects of osmanthus fragrans

It is well known that plant polyphenols can provide neuroprotection against cerebral ischemia as well as neurodegenerative process [22]. Thus, protecting brain cells from free radical damage could provide therapeutical intervention for stroke and many neurodegenerative diseases [23]. In this communication, Lin et al. [24] described that the extract from Osmanthus fragrans, which contains polyphenols, shows potent anti-oxidative properties and exerts neuroprotective functions in primary neuronal cultures. These findings are quite significant since they suggest that Osmanthus fragrans may provide as the starting material for further development of therapeutic agents for various oxidative stress-related disorders.

The involvement of serotonin receptors in suanzaorentang-induced sleep alteration

Epidemiological surveys have shown that about 20 % of adults have suffered from moderate to severe insomnia [25]. Although there are many sedative-hypnotic medications, including benzodiazepines and non-benzodiazepines, available for insomnia, they all have some degree of adverse effects including addiction and dependence. In contrast, Suanzaorentang, a traditional Chinese herb remedy, has been used for insomnia relief in China without noticeable adverse effect although its mechanism remains unclear. Recently, it was reported that suanzaorentang may increase spontaneous sleep activity partially through the activation of γ-aminobutyric acid (GABA) type A receptor, but not the GABAB receptor in the brain [26]. In this communication, Yi et al. [27] described that administration of 5-HT1A antagonist (NAN-190), 5-HT2 antagonist (ketanserin) or 5-HT3 antagonist (3-(4-Allylpiperazin-1-yl)-2-quinoxalinecarbonitrile) blocked suanzaorentang-induced NREMS increase, suggesting that the effect of suanzaorentang may be mediated through serotonergic activation, in addition to GABAergic system. These findings will provide foundation for the development of a new class of therapeutic agent for insomnia treatment.

References

1. Vassalle M., Analysis of cardiac pacemaker potential using a "voltage clamp" technique. Am. J. Physiol. 210: 1335–1341, 1966.

2. DiFrancesco D., A new interpretation of the pace-maker current in calf Purkinje fibres. J. Physiol. (London) 314: 359–376, 1981.
3. Vassalle M., The vicissitudes of the pacemaker current I (Kdd) of cardiac purkinje fibers. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9182-2).
4. Vassalle M., Yu H. and Cohen I.S., The pacemaker current in cardiac Purkinje myocytes. J. Gen. Physiol. 106: 559–578, 1995.
5. Chan W.E. and Chen S.S., Downregulation of human immunodeficiency virus type 1 Gag expression by a gp41 cytoplasmic domain fusion protein. Virology 348: 418–429, 2006.
6. Chan W.E. and Chen S.S., The dominant-negative action of a fusion protein containing the cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 in virus replication. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9186-y).
7. Hsu E.C., Lin Y.C., Hung C.S., Huang C.J., Lee M.Y., Yang S.C. and Ting L.P., Suppression of hepatitis B viral gene expression by protein-tyrosine phosphatase PTPN3. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9187-x).
8. Pittenger M.F. and Martin B.J., Mesenchymal stem cells and their potential as cardiac therapeutics. Circ. Res. 95: 9–20, 2004.
9. Toma C., Pittenger M.F., Cahill K.S., Byrne B.J. and Kessler P.D., Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105: 93–98, 2002.
10. Nassiri S.M., Khaki Z., Soleimani M., Ahmadi S.H., Jahanzad I., Rabbani S., Sahebjam M., Ardalan F.A. and Fathollahi M.S., The similar effect of transplantation of marrow-derived mesenchymal stem cells with or without prior differentiation induction in experimental myocardial infarction. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9188-9).
11. Van Vliet V.N., Chafe L.L., Antic V., Schnyder-Candrian S. and Montani J.-P., Direct and indirect methods used to study arterial blood pressure. J. Pharmacol. Toxicol. Methods 44: 361–373, 2000.
12. Fritz M. and Rinaldi G., Influence of nitric oxide-mediated vasodilation on the blood pressure measured with the tail-cuff method in the rat. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9191-1).
13. Wang N., Sun Y.H., Liu J., Wu G., Su J.G., Wang Y.P. and Zhu Z.Y., Knock down of gfp and no tail expression in zebrafish embryo by in vivo-transcribed short hairpin RNA with T7 plasmid system. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9189-8).
14. Palmieri A., Pezzetti F., Brunelli G., Zollino I., Scapoli L., Martinelli M., Arlotti M. and Carinci F., Differences in osteoblast miRNA induced by cell binding domain of collagen and silicate-based synthetic bone. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9193-z).
15. Wang Q., Huang Y., Ni Y., Wang H. and Hou Y., siRNA targeting midkine inhibits gastric cancer cell growth and induces apoptosis involved caspase-3, 8, 9 activation and mitochondrial depolarization. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9192-0).
16. Ryan A.K. and Rosenfeld M.G., POU domain family values: flexibility, partnerships, and developmental codes. Genes Dev. 11: 1207–1225, 1997.
17. Skotheim R.I., Lind G.E., Monni O., Nesland J.M., Abeler V.M., Fossa S.D., Duale N., Brunborg G., Kallioniemi O., Andrews P.W. and Lothe R.A., Differentiation of human embryonal carcinomas in vitro and in vivo reveals expression profiles relevant to normal development. Cancer Res. 65: 5588–5598, 2005.
18. Cheng C.J., Wu Y.C., Shu J.A., Ling T.Y., Kuo H.C., Wu J.Y., Chang E.E., Chang S.C. and Huang Y.H., Aberrant expression and distribution of the OCT-4 transcription factor in seminomas. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9198-7).
19. Nilsson G.E. and Lutz P.L., Release of inhibitory neurotransmitters in response to anoxia in turtle brain. Am. J. Physiol. 261(30) R32–R37, 1991.
20. Milton S.L., Thompson J.W. and Lutz P.L., Mechanisms for maintaining extracellular glutamate levels in the anoxic turtle striatum. Am. J. Physiol. 282: R1317–R1323, 2002.
21. Thompson J.W., Prentice H.M. and Lutz P.L., Regulation of extracellular glutamate levels in the long-term anoxic turtle striatum: coordinated activity of glutamate transporters, adenosine, K + ATP channels and GABA. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9190-2).
22. Yazawa K., Kihara T., Shen H., Shimmyo Y., Niidome T. and Sugimoto H., Distinct mechanisms underlie distinct polyphenol-induced neuroprotection. FEBS Lett. 580: 6623–6628, 2006.
23. Simonyi A., Wang Q., Miller R.L., Yusof M., Shelat P.B., Sun A.Y. and Sun G.Y., Polyphenols in cerebral ischemia: novel targets for neuroprotection. Mol. Neurobiol. 31: 135–147, 2005.
24. Lee H.-H., Lin C.-T. and Yang L.-L., Neuroprotection and free radical scavenging effects of *Osmanthus fragrans*. J. Biomed. Sci. 2007, (DOI 10.1007/s11373-007-9179-x).
25. Partinen M., Epidemiology of sleep disorders, In: Kryger M.H., Roth T. and Dement W.C. (Eds.), Principles and practice of sleep medicine. WB Saunders, Philadelphia, 1994, pp. 437–452.
26. Yi P.L., Tsai C.H., Chen Y.C. and Chang F.C., Gamma-aminobutyric acid (GABA) receptor mediates suanzaorentang, a traditional Chinese herb remedy, -induced sleep alteration. J. Biomed. Sci. 14: 285–297, 2007.
27. Yi P.L., Lin C.P., Tsai C.H., Lin J.G. and Chang F.C., The involvement of serotonin receptors in suanzaorentang-induced sleep alteration. J. Biomed. Sci. 14: 2007, (DOI 10.1007/s11373-007-9197-8).