P293

Involvement of Mechanosensitive Channels TRPV1, TRPV4, and ASIC3 at Zusanli (ST36) Acupoint : Possible Acupuncture Responding Channels?

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Backgrounds:

Acupuncture is an ancient therapy used for over thousands of years and gained scientific approval recently. It involves mechanostimulation after needling at acupoints. Although many proposed theories as endomorphin release or neural inhibition by adenosine after local ATP release, the link between mechanostimulation and following biological response is limited. TRPV1, TRPV4 and ASIC3 are suggested as mechanosensitive channels. Therefore we seek if they are involved during acupuncture.

Materials and Methods:

We compared Zusanli (ST36) acupoint with non-acupoint using western blot. Effects of manual acupuncture and agonist injection at ST36 on CFA inducted mouse were tested by withdraw time of radial heat.

Results:

TRPV1 was abundant in muscle and connective tissue of ST36, whereas TRPV4 and ASIC3 showed abundance at connective tissue. Second, TRPV1 agonist injected show similar effect as manual acupuncture.

Conclusion:

Our results revealed TRPV1, TRPV4, and ASIC3 were anatomically abundant at ST36 and TRPV1 resulted in similar acupuncture analgesic effect when stimulated. Interestingly, TRPV1, TRPV4, and ASIC3 can ATP release after stimulation. Thus, we suggest neuronal response to acupuncture is mediated directly by TRPV1 activation or indirectly by ATP release from tissues nearby. We stress the possibility of TRPV1, TRPV4 and ASIC3 as acupuncture responding channels. This can lead to create a new therapy by agonist injection and to the physiological identity of acupoints.

P294

Effects of Advanced Glycati… Stimulated Hypoxia Induced … Diabetic Retinopathy

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Backgrounds:

To investigate the molecular mechanism underlying AGEs-stimulated HIF-1α expression in diabetic retinopathy.

Materials and Methods:

In vitro, we treatment the human retinal pigment epithelial (RPE) cells with different dose AGEs. The expression of HIF-1α, PHD1, PHD2, PHD3, VHL and RAGE were examined by western blotting in RPE cells. Both RAGE and HIF-1α gene expression were verified by q-RTPCR. Different kinases inhibitors were evaluated to figure out the downstream signaling pathway that AGEs-stimulated HIF-1α. *In vivo*, Deposition of AGEs and expression of RAGE and HIF1α will be examined by immunohistochemical/ immunofluorescence staining in the retina of *db/db* mice, a diabetic animal model.

Results:

Our preliminary data implied that AGEs could significantly promote human retinal pigment epithelial (RPE) cell migration but not alter the proliferation. Interestingly, in normoxia condition, AGEs can enhance the HIF-1α protein expression in a dose and time dependent manners in RPE cells. The phosphorylation of Akt and p70S6K are involved AGEs-induced HIF1α expression and the pretreatment with the PI3K inhibitor (LY294002) and the p70S6K inhibitor (Rapamycin) can significantly reverse the phenomenon. AGEs also induced RAGE expression in a dose dependent manner in RPE cells. However, AGEs do not alter the HIF-1α mRNA expression and the PHD1, PHD2, PHD3 and VHL protein expressions. We also examined different kinds of AGEs including *N*-(carboxymethyl)lysine (CML) as well as methylglyoxal (MGO) and found that both AGEs can markedly induced HIF-1α protein expression in RPE cells.

Conclusion:

Our study revealed the AGEs can enhance the HIF-1α protein expression through the RAGE and the PI3K/Akt/p70S6K pathways and it may provide an important pathogenic molecular mechanism for AGEs in diabetic retinopathy.

P295

Effects of Natural Herbal Extracts and Their Components on Cardiac Disorders of Metabolic Syndrome Animal Models

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Backgrounds:

To investigate whether anytrocyanin and *Andrographis paniculata* extracts could inhibit cardiac cell apoptosis in diabetes and obesity respectively.

Materials and Methods:

Diabetes was induced in five-week-old male wistar rats using streptozotocin, then progressed for 1 weeks, and the treatment of extract product from purple rice, anthocyanins were gavage for 4 weeks constantly. Obesity was induced of four-week-old male C57/BL6 mice, high-fat diet by 45 kcal% were fed for ten months, and then *Andrographis paniculata* extracts were gavage for 1weeks. Moreover, cardiac diastolic and systolic function was assessed using ecocardiogaphy, and heart weight, cardiomyocyte morphology, protein level were also assessed individually.

Results:

As a result, anthocyanins and *Andrographis paniculata* extracts both significantly inhibited Fas-dependent and mitochondria-dependent apoptotic protein activation, prevented cardiomyocyte disarray and even restored cardiac function of diabetes and obesity animal hearts. Moreover, the progression of heart failure is through pathological hypertrophy to cause cardiomyocytes apoptosis, then lead to cardiac fibrosis, finally cause cardiac contractile dysfunction in diabetes and obesity animal hearts. However, anthocyanins and *Andrographis paniculata* extracts both reversed the heart damage effects, all the results were identified by western blot assay and histopathological analysis in the hearts of diabetes and obesity animal.

Conclusion:

Metabolic Syndrome may cause cardiomyocytes apoptosis and lead to fibrosis and cardiac dysfunction, but gavage natural herbal extracts anthocyanins and *Andrographis paniculata* prevented all those unhealthy effect of diabetes and obesity hearts.

P296

Behavioral Effects on Cue-dependent Fear Conditioning-Extinction process and Pre-synaptically Monoaminergic Changes within Fear Circuit in an Animal Model of Posttraumatic Stress Disorder

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Purpose:

Posttraumatic stress disorder (PTSD) is characterized by dysfunction of fear conditioning-extinction mechanism and abnormality of monoaminergic systems within the areas of fear circuit. Correlated exploration of behavioral and monoaminergic changes after extreme stress may provide useful evidence toward more understanding of the neurobiology of PTSD. The present study aimed to investigate the changes of fear conditioning-extinction mechanism and clarify the relationship of pre-synaptic monoamine neurotransmitters concentration, release capacity, and reuptake mechanism after single-prolonged stress (SPS).

Materials and Methods:

Rats were randomly divided into SPS and control groups. Locomotor activity prior to SPS was used as an index of rat's baseline performance. The degree of freezing was used to assess the cue-dependent fear conditioning-extinction after SPS. Further, HPLC with electrochemical detection techniques was used to examine both tissue and extracellular levels of dopamine (DA), norepinephrine (NE), serotonin (5-HT), and their metabolites in infralimbic cortex (IL), ventral hippocampus (vHPC) and basolateral of amygdala (BLA). Finally, the expression of DA transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT) level

Results:

SPS in the present study increased the locomotor activity and stimulus sensitivity and impaired the fear extinction function in cue-dependent fear conditioning. Pre-synaptic DA releasing was found specifically reduced in IL and vHPC. Furthermore, the expression of several monoamine transporters was changed in fear circuit after SPS.

Conclusion:

Pre-synaptically monoaminergic disruptions were involved in the mechanism of Cue-dependent Fear Conditioning-Extinction process after SPS.