

Supplementary Data

Brain Cholinergic Markers and Tau Phosphorylation are Altered in Experimental Type 1 Diabetes: Normalization by Electroacupuncture

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Accepted 29 August 2012

ELECTROACUPUNCTURE TREATMENTS

Electroacupuncture (EA) is a powerful form of sensory stimulation, activating type A and C sensory nerve fibers, with subsequent modulation of local, segmental, and central activity of the nervous system [1]. Rats belonging to EA groups were sedated superficially with an i.p. injection of chloral hydrate (200 mg/kg; Sigma-Aldrich S.r.l., Italy) and exposed to a 30-min session of EA twice a week for 3 weeks [2]. Stimulations were applied bilaterally at the traditional acupoint Zusanli (Stomach 36: St36), located on the antero-lateral side of the leg close to the anterior crest of the tibia. The point is innervated by the lateral sural cutaneous, the

cutaneous branch of the saphenous, and the deep peroneal nerves [3]. Stimulation of ST36 is effective in modulating peripheral NGF activity in the STZ model [2] as well as in eliciting a neurotrophin-mediated central response [4, 5]. Stimulation of ST36 in Alzheimer's disease patients has been also correlated with the activation of selected brain areas [6]. The needles (Hegu AB, Sweden) were inserted to depths of 0.3–0.5 cm and then attached bilaterally via clips electrodes to an electrical stimulator (ACUS II, Cefar, Sweden). Each pair of electrode/needles was inserted at the same point bilaterally. The points were stimulated with a low burst frequency of 2 Hz; each pulse was a square electric wave and had duration of 180 μ s, length of 0.1 s, and internal burst frequency of 80 Hz. The intensity (1.0–1.5 mA) was monitored by checking for local muscle contractions to reflect the activation of muscle-nerve afferents. The location and type of stimulation were the same in all rats. Control and STZ rats were manipulated and anaesthetized in a manner similar to the STZ + EA rats.

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Supplemental Table 1
Summary of ANOVA results

Protein	Tissue	Nr. of groups	df		F-value	P-value
			Treatment	Residual		
NGF	Cx	4	3	28	38.0	<0.0001
	Hp				13.0	<0.0001
TrkA	MS	3	2	21	6.1	0.0084
ChAT	MS				17.4	<0.0001
AD2	Cx	4	3	28	38.0	<0.0001
	Hp				9.3	0.0002
AT8	Cx				15	<0.0001
	Hp				35	<0.0001
p262	Cx				20	<0.0001
	Hp				4.2	0.0139
p231	Cx				3.5	0.0270
	Hp				3.8	0.0215
p-GSK3 β	Cx				47	<0.0001
	Hp				12	<0.0001
p-p38	Cx				18	<0.0001
	Hp				5.5	0.0042

One-way ANOVA has been performed for evaluation of: NGF (ELISA); TrkA and ChAT (automated cell count); tau phosphorylation epitopes AD2, AT8, p262 and p231 (western blot); phospho-GSK3 β and phospho-p38 kinases (western blot). $n = 8$ for each experimental group. Cx: Cortex. Hp: Hippocampus. MS: medial septum.

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