

## Potential Dietary Supplement Applications for Acetyl-L-Carnitine Arginate Dihydrochloride (ArginoCarn®)

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### **ArginoCarn® as a Dietary Support in Human Bowel Health**

**Akisu, M., D. Ozmen, et al. (2002). "Protective Effect of Dietary Supplementation with L-Arginine and L-Carnitine on Hypoxia/Reoxygenation-Induced Necrotizing Enterocolitis in Young Mice." *Biol Neonate* 81(4): 260-265.**

Oxygen-derived free radicals are important components of gastrointestinal injury in necrotizing enterocolitis (NEC). In the present investigation, we examined the protective actions of L-arginine, a nitric oxide synthase substrate, and L-carnitine against hypoxia-reoxygenation (H/R) induced NEC in young mice. Young mice were divided into four groups: group 1 mice were subjected to H/R only; group 2 H/R mice were supplemented with L-arginine in the drinking water (2 g/l) for 7 days; group 3 H/R mice were given L-carnitine solution in water (50 mg/kg p.o.) for 7 days, and group 4 mice served as controls. Hypoxia was induced by placing the mice in a 100% CO(2) chamber for 5 min. After hypoxia, the mice were reoxygenated for 10 min with 100% oxygen. We examined the intestinal lesions by light microscopy and measured the intestinal generation of thiobarbituric acid reactive substances (TBARS) and the activities of superoxide dismutase and catalase in the H/R-induced model of NEC. In both L-arginine and L-carnitine groups, the NEC-induced intestinal tissue damage was greatly attenuated, with necrosis limited partially to the mucosa. The tissue TBARS level was significantly higher in group 1 than in any of the other groups ( $p < 0.001$ ). However, those treated with L-arginine and L-carnitine had TBARS levels similar to those in the control animals. An increased tissue concentration of nitrate, a stable metabolite of nitric oxide, was found in the L-arginine-supplemented group as compared with the control group ( $p < 0.05$ ). Both superoxide dismutase and catalase activities in the intestine were similar in H/R groups when compared with the intestine of control animals. The present study suggests that oxygen-derived free radicals are involved in the pathogenesis of H/R-induced NEC. This study also shows that dietary supplementation with L-arginine and L-carnitine ameliorates the histological evidence of H/R-induced intestinal injury and significantly decreases lipid peroxidation in H/R-induced bowel injury. Based on these findings, the beneficial effects of L-arginine and L-carnitine in this model may be mediated via mechanisms preventing free radical damage.

**Kabaroglu, C., M. Akisu, et al. (2005). "Effects of L-Arginine and L-Carnitine in Hypoxia/Reoxygenation-Induced Intestinal Injury." *Pediatr Int* 47(1): 10-14.**

**BACKGROUND:** This study was designed to show the role of oxidative stress, nitric oxide and glutathione-related antioxidant enzymes in hypoxia/reoxygenation (H/R)-induced intestinal injury model in mice and to evaluate the potential benefits of arginine and carnitine supplementation. **METHODS:** A total of 28 young Balb/c mice were divided into four groups: Group 1 (untreated) was given physiological saline before the

experiment; group 2 H/R mice were supplemented with L-arginine; group 3 H/R mice were given L-carnitine for 7 days; and group 4 mice served as controls. At the end of day 7, H/R injury was induced and intestinal tissue malondialdehyde (MDA), nitrate levels and glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (GST) activities were measured. RESULTS: MDA levels were higher in the untreated animals than in the other three groups. MDA levels were higher in the L-arginine-treated animals than in the L-carnitine-treated animals. Nitrate levels were found to be increased in the L-arginine-treated group when compared to the controls. GSH-Px and GR activities were increased in the untreated, the L-arginine and the L-carnitine-treated H/R groups when compared to the control group. GST activities were indifferent between the groups. CONCLUSIONS: Oxidative stress contributes to the pathogenesis of H/R-induced intestinal injury. The glutathione redox cycle may have a crucial role in the H/R-induced intestinal injury. L-arginine and L-carnitine supplementations ameliorate the histological evidence of H/R-induced intestinal injury and decrease lipid peroxidation but do not alter the glutathione-related antioxidant enzyme activities.

**ArginoCarn® as a Dietary Support in Human Kidney Health**  
**Korish, A. A. (2009). "Oxidative Stress and Nitric Oxide Deficiency in Inflammation of Chronic Renal Failure. Possible Preventive Role of L-Arginine and Multiple Antioxidants." *Saudi Med J* 30(9): 1150-1157.**

OBJECTIVE: To evaluate the effect of L-arginine and multiple antioxidants on the inflammatory cytokines level, renal functions, blood pressure and dyslipidemia in chronic renal failure (CRF) rats. METHODS: This study was carried out between December 2007 and November 2008 in the Department of Physiology, Faculty of Medicine, King Saud University, Kingdom of Saudi Arabia. Chronic renal failure was induced in 40 rats by renal mass reduction (RMR) and 10 rats were sham operated. Renal mass reduction rats were treated for 12 weeks by L-arginine and/or a mixture of antioxidants (L-carnitine, Catechin, Vitamins E and C) and the effect of the treatments on plasma cytokines, soluble intercellular adhesion molecule-1 (sICAM-1), nitrate (NO<sub>2</sub>) and nitrites (NO<sub>3</sub>), lipid profile, blood pressure, and renal function was examined. RESULTS: Chronic renal failure increased plasma Interleukin (IL)-1alpha, IL-1beta, IL-6, tumor necrosis factor-alpha, soluble intercellular adhesion molecule-1 (sICAM-1) levels and decreased anti-inflammatory cytokines IL-4 and 10 levels. In addition, hypertension, and dyslipidemia were found. L-arginine treatment improved kidney functions, decreased systolic blood pressure and decreased inflammatory cytokines levels. Antioxidants administration decreased inflammatory cytokines and sICAM-1 levels and increased IL-4 levels. Combined use of L-arginine and the antioxidants mixture were very effective in their tendency to recover normal values of kidney functions, plasma cytokines, sICAM-1, blood pressure, NO<sub>2</sub>/NO<sub>3</sub>, cholesterol and triglycerides concentrations. CONCLUSION: Restoration of the pro-oxidant/ antioxidants balance with increased NO bio-availability counteracts inflammation, renal impairment and dyslipidemia in CRF. This may open new perspectives for the role of antioxidants and NO precursors in the treatment of uremia and its complications.

## **ArginoCarn® as a Dietary Support in Glucose Metabolism**

**Bloomer, R. J., K. H. Fisher-Wellman, et al. (2009). "Effect of Oral Acetyl L-Carnitine Arginate on Resting and Postprandial Blood Biomarkers in Pre-Diabetics." *Nutr Metab (Lond)* 6: 25.**

ABSTRACT: BACKGROUND: Resting and postprandial oxidative stress is elevated in those with metabolic disorders such as diabetes. Antioxidant supplementation may attenuate the rise in oxidative stress following feeding. Therefore we sought to determine the effects of acetyl L-carnitine arginate (ALCA) on resting and postprandial biomarkers of glucose and lipid metabolism, as well as oxidative stress. METHODS: Twenty-nine pre-diabetic men and women were randomly assigned to either 3 g.day<sup>-1</sup> of ALCA (n = 14; 31 +/- 3 yrs) or placebo (n = 15; 35 +/- 3 yrs) in a double-blind design, to consume for eight weeks. Fasting blood samples were taken from subjects both pre and post intervention. After each fasting sample was obtained, subjects consumed a high fat, high carbohydrate meal and additional blood samples were taken at 1, 2, 4, and 6 hours post meal. Samples were analyzed for a variety of metabolic variables (e.g., glucose, HbA1c, lipid panel, C-reactive protein, nitrate/nitrite, and several markers of oxidative stress). Area under the curve (AUC) was calculated for each variable measured post meal, both pre and post intervention. RESULTS: ALCA, but not placebo, resulted in an increase in nitrate/nitrite (25.4 +/- 1.9 to 30.1 +/- 2.8 mumol.L<sup>-1</sup>) from pre to post intervention, with post intervention values greater compared to placebo (p = 0.01). No other changes of statistical significance were noted (p > 0.05), although ALCA resulted in slight improvements in glucose (109 +/- 5 to 103 +/- 5 mg.dL<sup>-1</sup>), HbA1c (6.6 +/- 1.1 to 6.2 +/- 1.2%), and HOMA-IR (3.3 +/- 1.3 to 2.9 +/- 1.2). AUC postprandial data were not statistically different between ALCA and placebo for any variable (p > 0.05). However, nitrate/nitrite demonstrated a moderate effect size (r = 0.35) for increase from pre (139.50 +/- 18.35 mumol.L<sup>-1</sup>.6 hr<sup>-1</sup>) to post (172.40 +/- 21.75 mumol.L<sup>-1</sup>.6 hr<sup>-1</sup>) intervention with ALCA, and the magnitude of decrease following feeding was not as pronounced as with placebo. CONCLUSION: Supplementation with ALCA results in an increase in resting nitrate/nitrite in pre-diabetics, without any statistically significant change in other metabolic or oxidative stress variables measured at rest or post meal.

## **ArginoCarn® as a Dietary Support in Human Blood Lipids**

**Murosaki, S., T. R. Lee, et al. (2007). "A Combination of Caffeine, Arginine, Soy Isoflavones, and L-Carnitine Enhances Both Lipolysis and Fatty Acid Oxidation in 3t3-L1 and Hepg2 Cells in Vitro and in Kk Mice in Vivo." *J Nutr* 137(10): 2252-2257.**

To develop an anti-obesity agent containing dietary components, we focused on the mechanisms that enhance both lipolysis and fatty acid oxidation. Caffeine and arginine

(CA), a nonselective adenosine-receptor antagonist and an inducer of lipolytic hormone, respectively, were used to stimulate lipolysis. Soy isoflavones and L-carnitine (SL), stimulators of carnitine palmitoyl transferase 1A and a cofactor for beta-oxidation of fatty acids, respectively, were used to enhance fatty acid oxidation. Effects of a combination of CA and SL (CASL) on lipid metabolism were studied in vitro and in vivo. During 3T3-L1 differentiation, lipid accumulation was significantly lower in cells treated with CASL (50 micromol/L, 1 mmol/L, 1 micromol/L, and 1 mmol/L, respectively) compared with each alone. Lipolysis was also significantly greater in 3T3-L1 adipocytes treated with CASL (50 micromol/L, 1 mmol/L, 10 micromol/L and 0.5 mmol/L, respectively) compared with each alone. In addition, treatment with higher concentrations of CASL (2 mmol/L, 1 mmol/L, 10 micromol/L, and 1 mmol/L, respectively) significantly enhanced beta-oxidation in HepG2 cells. The effects of CASL-containing diets (250 mg, 6 g, 200 mg, and 1.5 g/kg diet, respectively) were studied in vivo. When KK mice were food deprived for 48 h and subsequently refed a fat-free diet for 72 h, hepatic triglyceride (TG) accumulation was significantly lower in mice fed CASL compared with the control mice. In addition, after obese KK mice were fed a low-fat diet for 2 wk, adipose tissue weights were significantly lower in those fed CASL, but not CA or SL alone, compared with the control mice. Plasma and liver TG levels were also lower in mice fed CASL than in the control mice. These results suggest that CASL is effective for controlling obesity.

***Khedara, A., T. Goto, et al. (1999). "Elevated Body Fat in Rats by the Dietary Nitric Oxide Synthase Inhibitor, L-N Omega Nitroarginine." Biosci Biotechnol Biochem 63(4): 698-702.***

The influence of the dietary nitric oxide (NO) synthase inhibitor, L-N omega nitroarginine (L-NNA) on body fat was examined in rats. In experiment 1, all rats were fed with the same amount of diet with or without 0.02% L-NNA for 8 wk. L-NNA intake caused elevations in serum triglyceride and body fat, and reduction in serum nitrate (a metabolite of nitric oxide). The activity of hepatic carnitine palmitoyltransferase was reduced by L-NNA. In experiment 2, rats were fed for 8 wk with the same amount of diets with or without 0.02% L-NNA supplemented or not with 4% L-arginine. The elevation in body fat, and the reductions in serum nitrate and in the activity of hepatic carnitine palmitoyltransferase by L-NNA were all suppressed by supplemental L-arginine. The results suggest that lower NO generation elevated not only serum triglyceride, but also body fat by reduced fatty acid oxidation.

***Goto, T., S. Ohnami, et al. (1999). "Feeding the Nitric Oxide Synthase Inhibitor L-N(Omega)Nitroarginine Elevates Serum Very Low Density Lipoprotein and Hepatic Triglyceride Synthesis in Rats." J Nutr Biochem 10(5): 274-278.***

This study was conducted to study the influence of dietary L-N(omega)nitroarginine (L-NNA), a nitric oxide (NO) synthase inhibitor, on serum lipids and lipoproteins and on the activities of enzymes related to lipid metabolism in rats. Feeding rats a diet containing 0.2 g/kg L-NNA for 5 weeks elevated serum concentrations of triglyceride, cholesterol, phospholipid, and free fatty acid and reduced serum nitrate (an oxidation product of

NO). The elevation in serum triglyceride was mainly due to the elevation in very low density lipoprotein (VLDL) triglyceride. Contents of cholesterol and phospholipid in the VLDL fraction also were elevated by L-NNA. L-NNA treatment caused significantly higher activity of hepatic microsomal phosphatidate phosphohydrolase (the rate-limiting enzyme in triglyceride synthesis) and lower activity of hepatic carnitine palmitoyltransferase (the rate-limiting enzyme in fatty acid oxidation). Activities of hepatic enzymes responsible for fatty acid synthesis such as glucose-6-phosphate dehydrogenase, malic enzyme, and fatty acid synthase were unaffected by L-NNA. The activity of hepatic microsomal phosphocholine cytidyltransferase (the rate-limiting enzyme in phosphatidylcholine synthesis) was reduced significantly by L-NNA. Our results suggest that lower NO production caused the elevations in hepatic triglyceride synthesis by higher esterification of fatty acid and lower fatty acid oxidation, leading to an enrichment of VLDL triglyceride.

**ArginoCarn® as a Dietary Support in Human Breast Health**  
**Erbas, H., N. Aydogdu, et al. (2007). "Protective Role of Carnitine in Breast Cancer Via Decreasing Arginase Activity and Increasing Nitric Oxide." *Cell Biol Int* 31(11): 1414-1419.**

Breast cancer remains one of the most common types of cancer. High levels of arginase and ornithine in different carcinomas may indicate their relation to cancer. Carnitine is a cofactor required for the transformation of free long-chain fatty acids into acetyl-carnitines. We have examined the protective effect of carnitine and the possibility that it disturbs arginase-nitric oxide (NO) interaction. Histopathological examination, arginase activity, ornithine and NO levels were determined in tumour tissues. Mitotic cells significantly decreased in the treatment group. Tissue arginase activity and ornithine levels decreased significantly with carnitine. NO levels were significantly higher in the treatment group. One of the possible mechanisms of carnitine's protective role in tumour progression might be its promotion of NO. This mechanism could decrease the production of tumour-promoting agents, polyamines, and increase the production of NO, thereby exerting a protective effect on cancer development.

**ArginoCarn® as a Dietary Support in Weight Control**  
**Treber, M., J. Dai, et al. (2003). "Identification by Mutagenesis of Conserved Arginine and Glutamate Residues in the C-Terminal Domain of Rat Liver Carnitine Palmitoyltransferase I That Are Important for Catalytic Activity and Malonyl-Coa Sensitivity." *J Biol Chem* 278(13): 11145-11149.**

Carnitine palmitoyltransferase I (CPTI) catalyzes the conversion of long chain fatty acyl-CoAs to acylcarnitines in the presence of L-carnitine. To determine the role of the conserved glutamate residue, Glu-603, on catalysis and malonyl-CoA sensitivity, we separately changed the residue to alanine, histidine, glutamine, and aspartate. Substitution of Glu-603 with alanine or histidine resulted in complete loss of L-CPTI activity. A change of Glu-603 to glutamine caused a significant decrease in catalytic

activity and malonyl-CoA sensitivity. Substitution of Glu-603 with aspartate, a negatively charged amino acid with only one methyl group less than the glutamate residue in the wild type enzyme, resulted in partial loss in CPTI activity and a 15-fold decrease in malonyl-CoA sensitivity. The mutant L-CPTI with a replacement of the conserved Arg-601 or Arg-606 with alanine also showed over 40-fold decrease in malonyl-CoA sensitivity, suggesting that these two conserved residues may be important for substrate and inhibitor binding. Since a conservative substitution of Glu-603 to aspartate or glutamine resulted in partial loss of activity and malonyl-CoA sensitivity, it further suggests that the negative charge and the longer side chain of glutamate are essential for catalysis and malonyl-CoA sensitivity. We predict that this region of L-CPTI spanning these conserved C-terminal residues may be the region of the protein involved in binding the CoA moiety of palmitoyl-CoA and malonyl-CoA and/or the putative low affinity acyl-CoA/malonyl-CoA binding site.

***Khedara, A., T. Goto, et al. (1999). "Elevated Body Fat in Rats by the Dietary Nitric Oxide Synthase Inhibitor, L-N Omega Nitroarginine." Biosci Biotechnol Biochem 63(4): 698-702.***

The influence of the dietary nitric oxide (NO) synthase inhibitor, L-N omega nitroarginine (L-NNA) on body fat was examined in rats. In experiment 1, all rats were fed with the same amount of diet with or without 0.02% L-NNA for 8 wk. L-NNA intake caused elevations in serum triglyceride and body fat, and reduction in serum nitrate (a metabolite of nitric oxide). The activity of hepatic carnitine palmitoyltransferase was reduced by L-NNA. In experiment 2, rats were fed for 8 wk with the same amount of diets with or without 0.02% L-NNA supplemented or not with 4% L-arginine. The elevation in body fat, and the reductions in serum nitrate and in the activity of hepatic carnitine palmitoyltransferase by L-NNA were all suppressed by supplemental L-arginine. The results suggest that lower NO generation elevated not only serum triglyceride, but also body fat by reduced fatty acid oxidation.

***Iwasaki, K., K. Mano, et al. (1987). "An Anabolic State in the Heart Induced by Arginine Intubation." Biochem Int 14(1): 129-134.***

Using rat heart perfusion, we found that an anabolic state can be induced with a medium which includes glucose, carnitine, branched-chain amino acids and arginine after arginine intubation at a dose of 250 mg/kg body weight. It showed diminished levels of glutamine, glutamate, branched-chain oxoacids and phenylalanine (a marker of heart protein metabolism) release, reflecting anabolic changes occurring in the myocardium. While ornithine intubation caused a catabolic state in which the release of alanine and glutamate was increased but phenylalanine release was unchanged. This anabolic state may be a useful model providing for myocardial protection.

**Johnston, C. S., C. Corte, et al. (2006). "Marginal Vitamin C Status Is Associated with Reduced Fat Oxidation During Submaximal Exercise in Young Adults." *Nutr Metab (Lond)* 3: 35.**

BACKGROUND: Vitamin C is a cofactor in the biosynthesis of carnitine, a molecule required for the oxidation of fatty acids. A reduction in the ability to oxidize fat may contribute to the reported inverse relationship between vitamin C status and adiposity. To examine this possibility, we conducted a preliminary trial to evaluate the impact of vitamin C status on fat oxidation during submaximal exercise. METHODS: Fat energy expenditure was determined in individuals with marginal (n = 15) or adequate (n = 7) vitamin C status during a submaximal, 60-minute treadmill test. Subsequently, eight of the subjects with marginal vitamin C status completed an 8-week double-blind, placebo-controlled, depletion-repletion trial with submaximal exercise testing. RESULTS: Individuals with marginal vitamin C status oxidized 25% less fat per kg body weight during the treadmill test as compared to individuals with adequate vitamin C status. Fat oxidation during exercise was inversely related to fatigue ( $r = -0.611$ ,  $p = 0.009$ ). Vitamin C repletion of vitamin C depleted subjects (500 mg vitamin C/d) raised fat energy expenditure during exercise 4-fold as compared to depleted control subjects ( $p = 0.011$ ). CONCLUSION: These preliminary results show that low vitamin C status is associated with reduced fat oxidation during submaximal exercise. Low vitamin C status may partially explain the inverse relationship between vitamin C status and adiposity and why some individuals are unsuccessful in their weight loss attempts.

**ArginoCarn® as a Dietary Support in Neurological Health**

**Edwards, R. L., K. Moseley, et al. (2009). "Long-Term Neurodevelopmental Effects of Early Detection and Treatment in a 6-Year-Old Patient with Argininaemia Diagnosed by Newborn Screening." *J Inherit Metab Dis*.**

Newborn screening makes possible the early identification and treatment of asymptomatic ARG1-deficient patients; however, it is unknown whether early intervention prevents neurological insults. We identified a full-term Hispanic male infant with argininaemia by newborn screening with a serum arginine of 327 micromol/L (reference values 0-140); ARG1 was undetectable on enzyme assay. Sequence analysis of ARG1 revealed a heterozygous nonsense mutation, c.223A>T (p.K75X), and a novel heterozygous missense variant, c.425G>A (p.G142E). Dietary protein restriction began from age 3 months, with addition of sodium benzoate at 4 months, and carnitine from 14 months. For the past 6 years, his serum arginine concentrations were maintained between 268 and 763 micromol/L (reference values 10-140). He has normal development without spastic paraplegia, but with mild hepatomegaly and stable hepatic dysfunction. A full neurodevelopmental assessment was conducted at age 5 years. The BASC-2 rated the patient's behaviours as age-appropriate. The Leiter-R assessed his 'Fundamental Visualization', 'Sequential Order', and 'Picture Concept' at 'Average', 'Form Completion' and 'Matching' at 'Low Average', and 'Figure Ground' and 'Repeated Patterns' in the 'Deficit' range. The full-scale IQ and the functioning ability presented in the 'Borderline' range and in the 'Low Average' range, respectively. The VABS/Survey -

Spanish Version showed difficulty in receptive and written language and fine and gross motor skills, and his performance to be at younger than his chronological age. The Short Sensory Profile showed some difficulty with taste and smell sensitivity. Long-term observation over 6 years in a patient with early treated argininaemia shows promising neurodevelopmental results.

**Scorziello, A., O. Meucci, et al. (1997). "Acetyl-L-Carnitine Arginine Amide Prevents Beta 25-35-Induced Neurotoxicity in Cerebellar Granule Cells." Neurochem Res 22(3): 257-265.**

Cerebellar granule cells (CGC) at different stages of maturation in vitro (1 or 6 DIV), were treated with beta 25-35 and acetyl-L-carnitine arginine amide (ST857) in presence of 25 mM KCl in the culture medium, and neuronal viability was assessed. Three days of treatment slightly modified the survival of 1 DIV-treated cells, which degenerate and die five days later beta-amyloid matching. Similarly, a significative neurotoxic effect was observed on 6 DIV treated-cells after 5 days of exposure to the peptide, while the death occurred within 8 days. ST857 coincubated with beta 25-35 was able to rescue neurons from beta 25-35-induced neurotoxicity. We also studied the changes in Ca<sup>2+</sup> homeostasis following glutamate stimulation, in control and beta-amyloid treated single cells, either in presence or in absence of ST857. beta 25-35 did not affect basal [Ca<sup>2+</sup>]<sub>i</sub>, while modified glutamate-induced [Ca<sup>2+</sup>]<sub>i</sub> increase, causing a sustained plateau phase of [Ca<sup>2+</sup>]<sub>i</sub>, that persisted after the removal of the agonist. ST857 pretreatment completely reverted this effect suggesting that, in CGC chronically treated with beta 25-35, ST857 could protect the cells by neurotoxic insults of the peptide likely interfering with the cellular mechanisms involved in the control of Ca<sup>2+</sup> homeostasis.

**Tagliatela, G., D. Navarra, et al. (1995). "Neurite Outgrowth in Pc12 Cells Stimulated by Acetyl-L-Carnitine Arginine Amide." Neurochem Res 20(1): 1-9.**

Senescence of the central nervous system is characterized by a progressive loss of neurons that can result in physiological and behavioral impairments. Reduction in the levels of central neurotrophic factors or of neurotrophin receptors may be one of the causes of the onset of these degenerative events. Thus, a proper therapeutic approach would be to increase support to degenerating neurons with trophic factors or to stimulate endogenous neurotrophic activity. Here we report that acetyl-L-carnitine arginine amide (ST-857) is able to stimulate neurite outgrowth in rat pheochromocytoma PC12 cells in a manner similar to that elicited by nerve growth factor (NGF). Neurite induction by ST-857 requires de novo mRNA synthesis and is independent of the action of several common trophic factors. The integrity of the molecular structure of ST-857 is essential for its activity, as the single moieties of the molecule have no effect on PC12 cells, whether they are tested separately or together. Also, minor chemical modifications of ST-857, such as the presence of the arginine moiety at a position other than the amino one, completely abolish its neuritogenic effect. Lastly, the presence of ST-857 in the culture medium competes with the high affinity NGF binding in a dose dependent fashion. These results, although preliminary, are suggestive of a possible role for ST-857 in the development of therapeutic strategies to counteract degenerative diseases of the CNS.

**Tewari, K., J. M. Simard, et al. (1995). "Acetyl-L-Carnitine Arginyl Amide (St857) Increases Calcium Channel Density in Rat Pheochromocytoma (Pc12) Cells." *J Neurosci Res* 40(3): 371-378.**

We used the patch clamp technique to study the effect of acetyl-L-carnitine arginyl amide (ALCAA) and of nerve growth factor (NGF) on availability of L-type Ca<sup>2+</sup> channels in rat pheochromocytoma (PC12) cells maintained in defined medium. Channel availability was measured as number of channels in the patch x the probability of opening (n.Po). In patches from control cells, cells exposed to NGF (10 ng/ml) for six days, and cells exposed to ALCAA (1 mM) for six days, n.Po, measured during 200-240 ms pulses to -10 mV (holding potential, -60 mV), was 0.102 +/- 0.089 (5 cells), 0.173 +/- 0.083 (5 cells), and 0.443 +/- 0.261 (7 cells), respectively. The 4.3-fold increase for the ALCAA-treated cells was significantly different from control (P < 0.05), whereas that for the NGF-treated cells was not. For the same conditions, the maximum number of superimposed openings at -10 mV was 1.3 +/- 0.5 (6 cells), 1.6 +/- 0.5 (8 cells), and 3.3 +/- 1.8 (8 cells), with the value for the ALCAA-treated cells being significantly different from control (P < 0.001). Additional analysis showed that the distribution of channel open times, the time constants, and the voltage dependence of activation were not changed by prolonged exposure to ALCAA. Short-term exposure to both ALCAA as well as to the parent compound, acetyl-L-carnitine (ALCAR), did not cause an increase but rather a decrease in n.Po, and this short-term effect of both compounds was blocked by neomycin, an inhibitor of phospholipase C.

**Westlund, K. N., Y. Lu, et al. (1992). "Effects of Nerve Growth Factor and Acetyl-L-Carnitine Arginyl Amide on the Human Neuronal Line Hcn-1a." *Int J Dev Neurosci* 10(5): 361-373.**

The HCN-1A clonal cell line, derived from the cortical tissue of a patient with unilateral megencephaly, was shown to differentiate into a mature neuronal-like state in the presence of the nerve growth factor, dibutyryl cyclic adenosine, 3',5'-monophosphate and either 1-isobutyl-3-methylxanthine or forskolin. Differentiation was assessed by measuring the percentage of cells that displayed branched, varicose processes that stained for synaptophysin. Treatment of cultures with a cocktail containing forskolin increased immunocytochemical staining for gamma aminobutyric (GABA), neurofilament protein and the nerve growth factor receptor species p75NGFR. Treatment with acetyl-L-carnitine alone had some effects on the cell morphology while acetyl-L-carnitine arginyl amide and nerve growth factor together increased the GABA content. Positive staining levels for the neurotransmitters gamma aminobutyric acid, glutamate, somatostatin, cholecystinin and vasoactive intestinal polypeptide were measured quantitatively for HCN-1A under basal conditions.