Opioid Peptides Increase Blood Pressure and Enhance Survival of Rats Undergoing Hemorrhagic Shock without Fluid Resuscitation

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ABSTRACT

Rats weighing 300-350 g had catheters placed in the femoral artery (for hemorrhage), tail artery for blood pressure (BP) measurements and the tail vein (for administration of opioids) controls received saline or opioids without hemorrhage. For the moderate hemorrhage studies (5.5 ml hemorrhage volume) animals received saline or Deltorphin-D (Delt-D) a delta specific opioid receptor agonist prior to hemorrhage without fluid resuscitation and post-treated animals received saline or Delt-D 1 mg/kg or Delt-D 2 mg/kg following hemorrhage without fluid resuscitation. BP, blood loss and rectal temp, at beginning and end of hemorrhage were determined. The effect of Delt-D infusions on the expression of Ubiquitin B and C (UBB and UBC) was determined. Heat Shock Protein (HSP-70), and inducible Nitric Oxide Synthase (iNOS) mRNA transcripts in heart, leg and brain were determined after 2 hr. Preinfusions of Delt-D did not significantly effect BP while 2 mg/kg post-hemorrhage infusions without resuscitation fluid significantly increased BP compared to controls and decreased core temp by 4.5°F compared to controls. Delt-D infusions increased iNOS and HSP70 mRNA in heart and leg in non-hemorrhaged controls and UBB in brain of non-hemorrhaged controls. Pre-treated Delt-D animals had elevated brain iNOS and HSP70 mRNA, and post-hemorrhage Delt-D treated animals had elevated UBC mRNA in heart and brain and HSP70 mRNA in leg tissue. For the severe hemorrhage protocol (9.0 - 11.0 ml hemorrhage volume representing 53-61% of total blood volume), rats were infused with either 3.0 mg/kg of a highly specific mu opioid, (ZGI-06) or a Delt-D variant (ZGI-07) and ischemic tolerance (ie BP and 6 hr survival) was monitored. Controls were infused with l.0 ml PBS. Six hr survival was 33% for controls, 60% for ZGI-06 and 72% for ZGI-07, BP increased within 30-45 seconds after infusion of ZGI-06 by 29.5± 13.0 mmHg vs. controls –1.5 ± 19.4 mmHg while ZGI-07 increased BP by 38.8 ± 18.5 mmHg vs. control.

INTRODUCTION

The role of *delta*-specific opioids in providing multiorgan, myocardial and cerebral ischemia protection has been elucidated over the past 12 years. Evidence has accumulated that *delta* opioid

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agonists composed of two subtypes specific for *delta$_1$* and *delta$_2$* opioid receptor subtypes can confer myocardial ischemic preconditioning IPC and pharmacological (delayed) ischemic preconditioning (PPC) in dog multiorgan autoperfusion bloc,[1] in isolated rat[2-4], rabbit[5-7] and pig heart models [8] as well as in intact ischemic rat[9-10]and pig heart models[11], a mouse hypoxic model[12-13]and even in myocardial cell culture model[14]. It is also now known that universal opiate antagonists, naltrexone as well as specific *delta$_1$* and *delta$_2$* antagonists can block or retard both classical IPC and PPC in a dose dependent manner[14]. The predominance of *delta* opioid receptor mRNA transcripts in human myocardium has been recently in documented[15]. It has also been demonstrated that the IPC occurring in patients following two sequential angioplasty balloon inflations could be abolished following infusions of the universal opiate antagonist, naloxone[16]. Similarly infusions of the non-specific delta opioid, DADLE, into organ baths containing human trabeculae obtained during bypass surgery provided IPC as evidenced by their enhanced contractile force following ischemia[17]. The intermediary role of mitochondrial and sarcolemmal Delt-D), a highly specific *delta$_2$* opioid agonist, could provide PPC and reduce left ventricular infarct size in an intact ischemic pig heart model [8] when infused 45 min prior to ischemia. Delt-D is a 17 amino K_{ATP} channels in classical IPC and PPC has been documented in intact ischemic [10] and isolated[15] rat heart models and in myocardial cell cultures[18].

We have recently documented that infusions of DPDPE (D-Pen$^{2-5}$, Enkephalin), a highly specific *delta$_1$* opioid agonist, and Deltorphin-D (acid peptide originally isolated from skin secretions of the Brazilian frog *Phyllomedusa burmeisterie* [19]. The focus of the present study was (1) to determine in a moderate hemorrhage protocol (ie, 5.5 ml total hemorrhage volume) if infusions of a Delt-D (Delt-D) without any fluid resuscitation will enhance ischemic tolerance, blood pressure (BP), and alter the expression of mRNA transcripts of Ubiquitin B and C, Heat Shock Protein (HSP-70) and Inducible Nitric Oxide Synthase (iNOS) and (2) to determine in severe hemorrhage protocol (ie 9.5 to 11.5 ml) total hemorrhage volume if infusions of a ZGI-06 on a ZGI-07 without concomitant fluid resuscitation enhance ischemia tolerance (ie increase BP and 6 hr survival) compared to controls receiving PBS infusions.

MATERIALS AND METHODS

HEMORRHAGIC SHOCK MODEL

The hemorrhagic rat model was that of Summers et al.[21], where we used male Sprague Dawely rats weighing 300 to 350 mg. Catheters were placed into the femoral artery (for bleeding), femoral vein (for opiate injections) and tail artery (for BP measurements), and were brought underneath the skin to an incision at the back of the neck where they exited the body. Rats were hemorrhaged the day after catheterization, and during hemorrhage BP was allowed to drop to 40-50 mmHg.

Moderate Hemorrhage Protocol

Saline 0.5 ml or Delt-D at a concentration of either 1 or 2 mg/kg was administered at the end of the hemorrhage protocol (lasting about 15 min. and representing about 5.5 ml per rat blood loss which is approximately 30%of total blood volume). Rats were killed at 2 hr following UHS and tissues

(brain, heart and leg muscle) were collected for mRNA isolation and northern blot analysis for stress proteins (Ubiquitin, HSP-70 iNOS). Core temperature was monitored at the beginning and end of the experiment. The data collected included BP and mRNA data for the heart, leg and brain tissue using Ubiquitin (UBB and UBC), HSP70, and iNOS probes. Rats were randomly divided into 1 of 7 groups. The groups included: control –no hemorrhage saline (n=5), control no hemorrhage Delt-D l mg/kg (n=4), hemorrhage pretreatment saline (n=5), hemorrhage pretreatment Delt-D l mg/kg (n=6), hemorrhage post-treatment saline (n=6), hemorrhage post-treatment Delt-D 1 mg/kg (n=4) and hemorrhage Post-treatment Delt-D 2 mg/kg (n=5).

Severe Hemorrhage Protocol

Rats were hemorrhaged 9.5 to 11.0 ml representing 51- 60% of total blood volume. A highly specific *mu* opioid, ZGI-06 or ZGI-07, or PBS were infused (dissolved l.0 ml PBS pH 7.4) into the femoral vein over a 20-30 second interval when blood pressure declined to between 40-60 mmHg. Ischemic tolerance was measured (increased BP and 6 hr survival).

RESULTS

Moderate Hemorrhage Protocol

Pretreatment – Blood Pressure

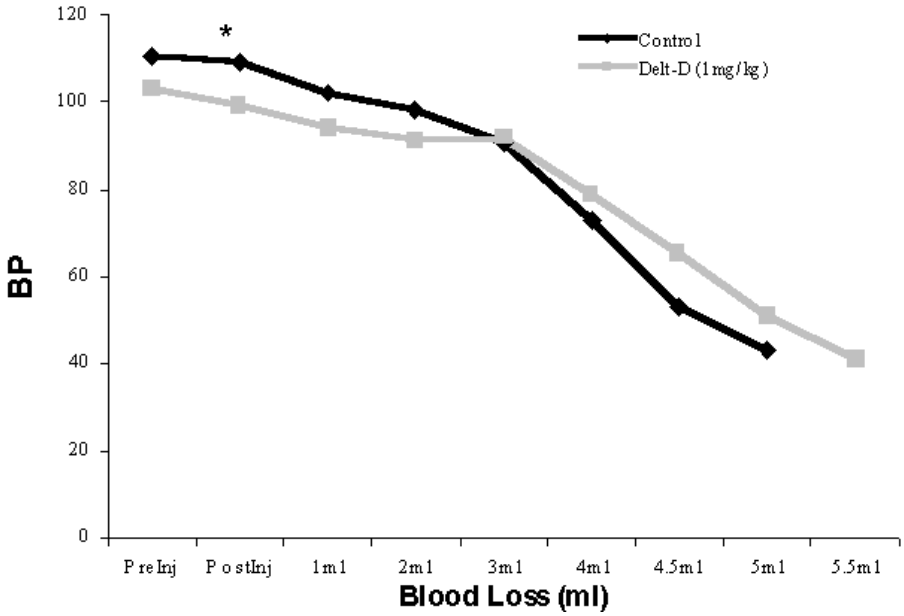

Figure 1: Pre-treatment BP values. No significant difference in BP noted between slopes of Saline Control and Deltorphin-D (Delt-D) groups.

Delt-D pretreatment had no significant effect on BP prior to hemorrhage (**Fig. 1).**

Post-treatment - Blood Pressure

Analysis of post-treatment 5 min slope data indicated significant differences between the 3 hemorrhage treatment group at the beginning of the recovery period (0-5 min), (11-15 min), (16-20 min) and near the end (46-50 min) in rats injected with Delt-D at a conc. of 2.0 mg/kg compared to controls but not at a conc of 1.0 mg/kg. (**Fig. 2**). In the heart tissue, UBC mRNA transcripts was significantly elevated in Delt D2 treated animals in comparison to controls, and iNOS and HSP70 mRNA in the heart of Delt-D – controls were significantly higher when compared to all other groups (**Fig. 3)**. The leg tissue was similar to the heart tissue in that animals receiving only Delt D1 (control) showed significant increases in iNOS and HSP70 compared to all other groups and the post-treatment Delt-D1 and Delt-D2 animals showed elevated HSP70 levels compared to all groups (control-no hemorrhage Delt D1) as seen in **Fig. 4**. In brain UBC mRNA transcripts were elevated in Delt-D treated animals and iNOS was elevated in pretreated saline and HSP70 and iNOS were elevated in the Delt-D pretreated group as seen in **Fig. 5**.

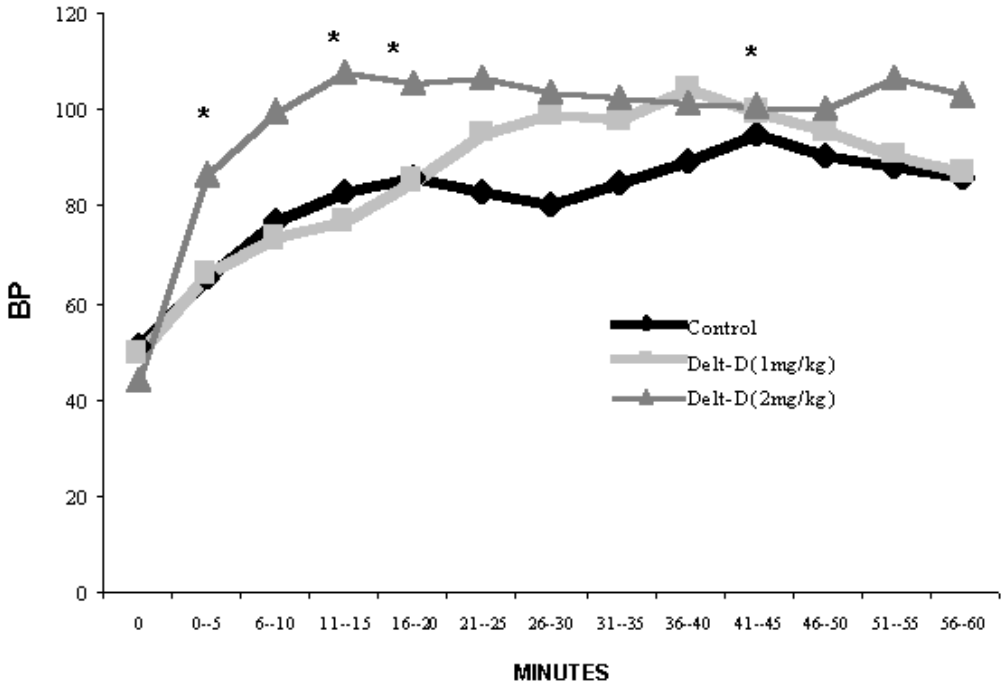

Figure 2: Post-treatment BP values, 5 min intervals. Significant differences in slopes between 3 groups observed in first 5 min (p≥0.05), at 11-15 min (p=0.04), 16-20 min (p=0.05) and 46-50 min (p≥0.05)

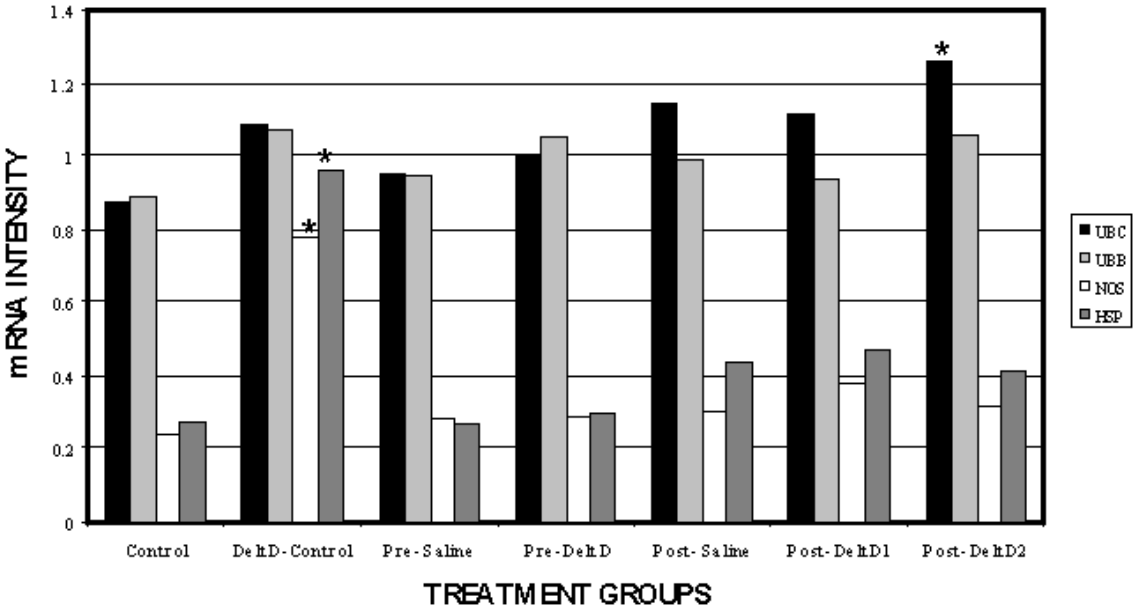

Figure 3: Heart mRNA. UBC was upregulated in Post-Delt-D2 animals compares to controls (p = 0.04). In Delt-D Control no hemorrhage group iNOS and HSP70 significantly increased (p ≥ 0.05)

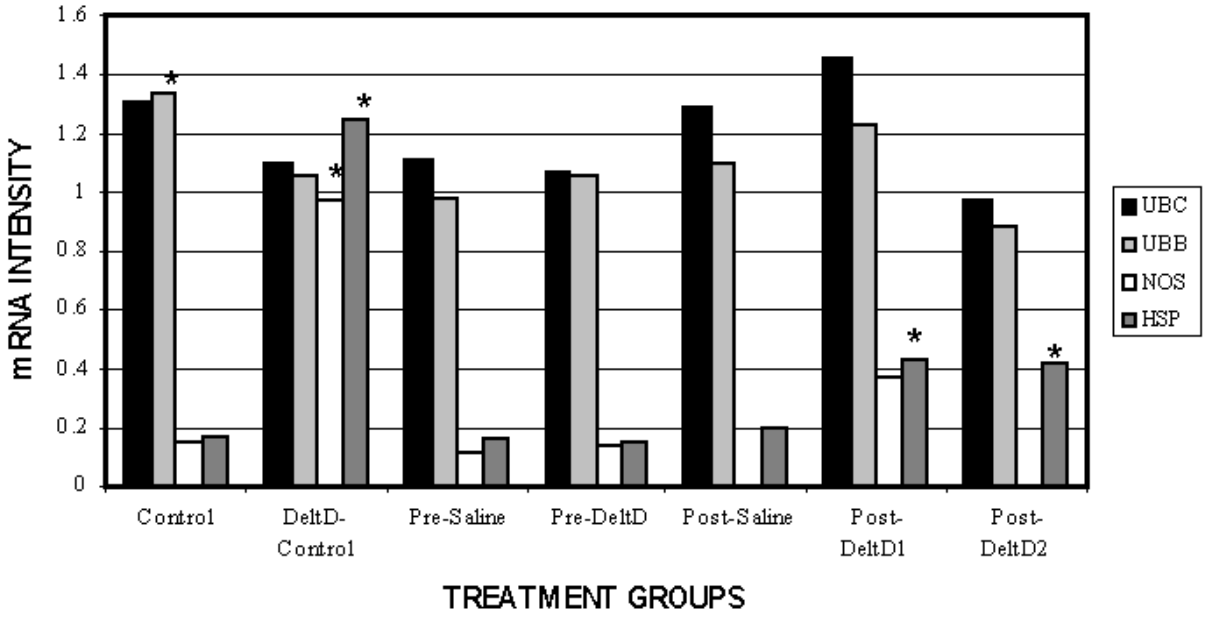

Figure 4: Leg mRNA. UBB but not UBC upregulated in controls compared to post-treatment Delt-D2 (p=0.04). Delt-D control no hemorrhage had significantly increased iNOS and HSP70 compared to all other groups (p≥ 0.05). Post-tratment Delt-D1 and Delt-D2 had significantly elevated HSP70 compared to all groups except Delt-D control (p≥ 0.05).

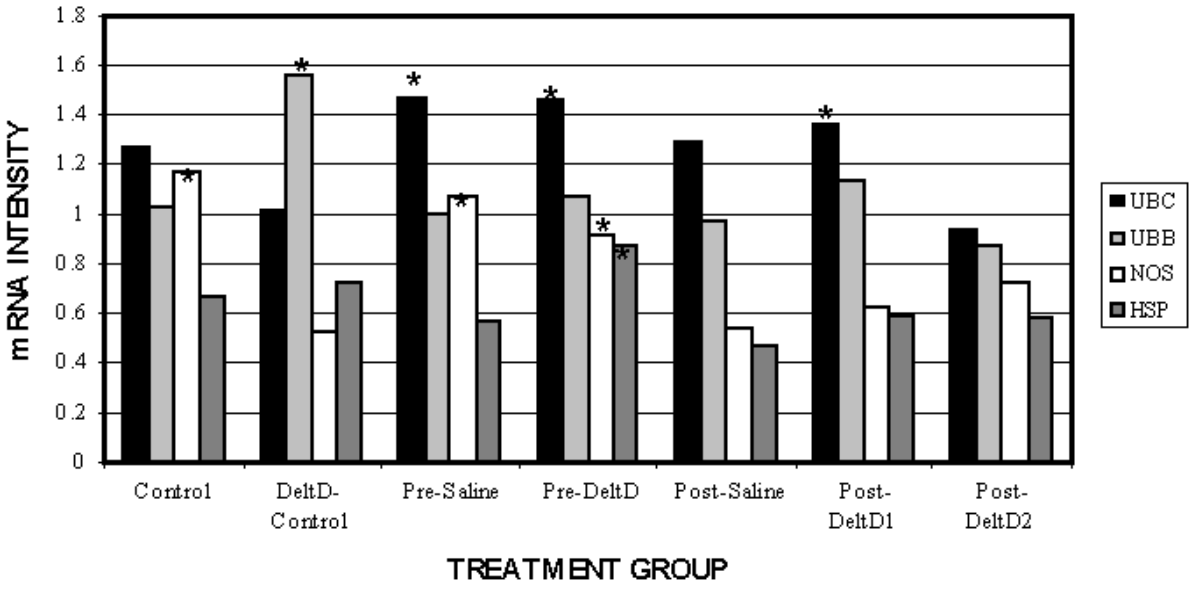

Figure 5: Brain mRNA. In brain significant increases in UBC in animals pre-treated saline or pre-Delt-D compared to non-hemorrhaged control (p≥ 0.05). Post-treatment Delt-D2 UBC lower than all other hemorrhage groups (p≥ 0.05). In brain non-hemorrhaged saline control and pre-treatment haemorrhaged saline and Delt-D groups had significantly higher values iNOS (p≥ 0.05).

Severe Hemorrhage Protocol

Hemorrhage (9.0-11.0 ml representing 51-60% of total blood volume) resulted in the following: BP increased by 29.5 ± 13.0 mmHg for ZGI-06 infused rats (n=11) within 30-45 sec following infusion while controls (n=6) decreased by 1.5 ± 19.5 mmHg (p=0.01). ZGI-07 infused rats (n=11) increased BP by 38.8 ± 18.5 mmHg vs controls (p=0.002) as seen in **Figure 6**. Six hour survival for controls was 33% (n=2), 54% (n=6) for ZGI-06 and 72% (n=8) for ZGI-07.

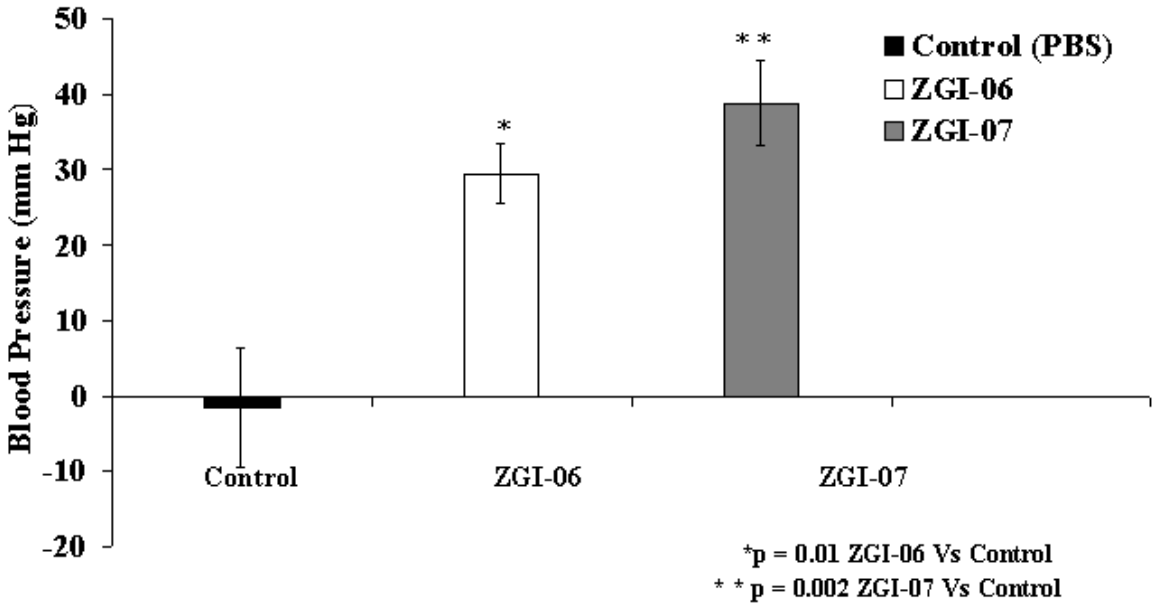

Figure 6: Effect of opioids on Blood Pressure Following Severe Hemorrhage

CONCLUSIONS

Moderate Hemorrhage Protocol

1) Pre-hemorrhage Delt-D treatment does not significantly alter BP compared to saline controls.

2) Delt-D at 2 mg/kg increases BP vs saline controls during the lst hour following hemorrhage **without fluid resuscitation.**

3) Following hemorrhage Delt-D treated animals had 4.5°F decrease in temperature compared to saline treated controls.

4) In Heart, UBC mRNA levels were significantly elevated in Delt-D treated animals, following hemorrhage.

5) In the brain, Delt-D pretreated animals had up regulated levels of UBC and HSP70, UBC and HSP70 which are thought to be involved in cyto protection during times of stress (i.e. hemorrhage). Also endogenous opioid system may be involved in modulating peripheral nervous system during hemorrhage which would directly effect BP and heart rate (Molina*, Clin. Exp Pharm Physiol* 29(3) 248, 2002).

6) In the heart and brain, post treated Delt-D animals had enhanced levels of UBC mRNA compared to controls.

7) In both heart and skeletal muscle, iNOS mRNA levels were lower compared to controls iNOS is over produced in tissues during extreme stress and has been implicated in tissue damage and death.

8) In skeletal muscle, HSP70 mRNA was elevated in post-hemorrhage treated animals. Previous studies have shown that HSP70 regenerates denatured protein in skeletal muscle.

Severe Hemorrhage Protocol

9) Six-hour survival was 33% for controls 54% for ZGI-06 and 72% for ZGI-07.

10) ZGI-06 increased BP by 29.5 ± 13 mmHg vs control –l.5 ± l9.5 mmHg (p=0.01) and ZGI-07 increased BP by 38.8 ± 18.5 mmHg vs control within 30-45 seconds after infusion.

11) *Delta* and *mu* specific opioids increase BP and 6hr survival in severely hemorrhaged rats without concomitant fluid resuscitation.

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