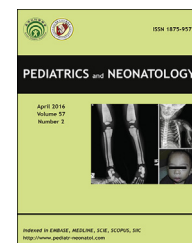


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## ORIGINAL ARTICLE

# Calcitonin Gene-Related Peptide Downregulates Expression of Inducible Nitride Oxide Synthase and Caspase-3 after Intestinal Ischemia-Reperfusion Injury in Rats

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## Key Words

calcitonin gene-related peptide;  
caspase-3;  
inducible nitride oxide synthase;  
ischemia-reperfusion injury;  
small intestine

**Background:** Various investigations have demonstrated that calcitonin gene-related peptide (CGRP) plays an important role in mediating ischemic preconditioning. CGRP has been shown to mimic the protective effects of ischemic preconditioning and mitigate ischemia-reperfusion (I/R) injury in the heart, brain, gastrointestinal system, and other tissues. This study aimed to examine whether CGRP, a proven intestinal cytoprotective molecule, exerted its protective effects through modulation of inducible nitride oxide synthase (iNOS) and apoptosis after intestinal I/R injury.

**Methods:** This animal study randomly divided 30 rats into the following five groups: (1) the normal control group, (2) the ischemia group with normal saline, (3) the I/R group with normal saline, (4) the ischemia group with CGRP (300 μg/kg), and (5) the I/R group with CGRP (300 μg/kg). Levels of iNOS messenger RNA (mRNA) and protein, and caspase-3 protein were determined by real-time quantitative polymerase chain reaction and Western blotting analyses, respectively. Statistical analysis was performed using analysis of variance with Dunn test.

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**Results:** The mRNA levels of iNOS increased after the intestinal ischemia or intestinal reperfusion phase ( $p < 0.01$ ), and CGRP pretreatment significantly decreased iNOS mRNAs and protein levels ( $p < 0.01$ ). The expression protein levels of caspase-3 increased after the intestinal ischemia or intestinal reperfusion phase. CGRP pretreatment significantly decreased the levels of caspase-3 proteins. CGRP intestinal cytoprotection is mediated, in part, by downregulation of expression of iNOS and caspase-3 after intestinal I/R injury.

**Conclusion:** The study indicates that the cytoprotective role of CGRP (i.e., antiapoptotic effect) after I/R injury could be via downregulation of iNOS, which may relieve I/R tissue damage by blocking iNOS activity.

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## 1. Introduction

Intestinal ischemia-reperfusion (I/R) injury is known to be a key factor in the pathogenesis of various clinical conditions, such as midgut volvulus, neonatal necrotizing enterocolitis, intestinal transplantation, and hemorrhagic shock with resuscitation.<sup>1</sup> The precise mechanisms of intestinal I/R injury have not yet been fully elucidated, and there is currently no specific treatment once ischemia has occurred. However, more recent reports have noted that apoptosis is a significant and perhaps principal contributor to cell death after I/R injury.<sup>2,3</sup>

The development of intestinal injury following I/R appears to involve the endogenous production of inflammatory mediators such as platelet-activating factor (PAF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and other cytokines,<sup>2</sup> and increasing evidence suggests that the vasodilator NO may be involved in this process.<sup>4</sup> There is ample evidence that I/R injury is associated with inducible nitric oxide synthase (iNOS) and sustained NO production in several organs including the heart, brain, muscle, and pancreas.<sup>5</sup> Dramatic upregulation of iNOS is induced by inflammatory cytokines during intestinal injury, leading to a very rapid and quantitatively massive generation of NO, which participates in the generation of tissue injury.

*Calcitonin gene-related peptide* (CGRP) is a 37-amino acid peptide that is generated by tissue-specific alternative processing pathway of the calcitonin/CGRP gene.<sup>6</sup> CGRP exerts multiple biological effects on the central nervous, cardiovascular, and gastrointestinal systems.<sup>7</sup> CGRP plays an important role in mediating communications between the nervous, endocrine, and immune systems. CGRP has pleiotropic functions that have been implicated in the regulation of cell proliferation,<sup>8</sup> apoptosis,<sup>9</sup> and differentiation.<sup>10</sup> Recently, CGRP has been reported to exert a protective effect against organ damage in several disease models.<sup>11,12</sup> In a previous study, CGRP exerted a significant antiapoptotic effect on cultured hepatocytes treated with interferon- $\alpha$ . Furthermore, pretreatment with CGRP significantly inhibited TNF- $\alpha$ -induced apoptosis in cultured endothelial cells.<sup>13</sup>

This animal study evaluates the role of CGRP, a proven intestinal cytoprotective molecule, in I/R injury by regulating the expressions of iNOS and caspase-3.

## 2. Methods

### 2.1. Experimental protocol

After obtaining approval from the Chang Gung Memorial Hospital Animal Research and Ethics Committee, Taoyuan, Taiwan, adult, Sprague-Dawley rats (weighing 200–300 g; aged 8–12 weeks) were anesthetized with Chloralurat (Merk Ltd., Germany) (10%) intraperitoneally. Midline laparotomy was performed, the small intestine was reflected to the left, and a 10-cm segment of the distal isolated ileal loop was created. The marginal vessels were divided, resulting in complete separation of the vascular supply to the loop. The mesentery of the isolated loop was occluded for 30 minutes in experimental animals with a micro-bulldog clamp. At the end of the ischemic period, the clamp was released and the bowel became pink. Animal preparations and methods of surgery have been described in detail in a previous paper.<sup>14</sup> For treated animals, a dose of 300  $\mu\text{g}/\text{kg}$  of CGRP (Sigma-Aldrich, St Louis, MO) was given in a total volume of 1.5 mL of saline. The preparation was injected through the internal jugular vein. The injection was given 5 minutes before the initiation of ischemia.

Thirty rats were randomly divided into the following five groups: the normal control group ( $n = 6$ ) with sham operation; the disease group ( $n = 12$ ) including the ischemia group, receiving vascular occlusion for 30 minutes with normal saline; the I/R group, receiving vascular occlusion for 30 minutes and reperfusion for 30 minutes with normal saline; the disease experimental group including the ischemia group with CGRP (300  $\mu\text{g}/\text{kg}$ ); and the I/R group with (300  $\mu\text{g}/\text{kg}$ ). A total of 30 rats were used in this experiment.

### 2.2. Collection of samples

Rats were killed by intramuscular injection of ketamine. A 3-cm occluded ileum was then excised. The isolated ileum was gently rolled with cotton swabs and flushed with cold (4°C) normal saline to remove the luminal contents, blotted dry, weighed, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  for subsequent analysis.

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