ORIGINAL ARTICLE

Effects of CO_2 Pneumoperitoneum and Enteric Disturbance on β-endorphin in SD Rats

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Abstract

Objective: To study the effects of enteric disturbance and CO_2 pneumoperitoneum on serum β-endorphin (β-EP) in SD rats, and discuss their influences on perioperative stress responses.

Methods: 120 SPF-grade male SD rats were anesthetized intraperitoneally and equally randomized to four groups: group A, CO_2 pneumoperitoneum at 1.0 kPa; group B, a 5 cm abdominal incision without enteric disturbance; group C, a 5 cm abdominal incision with enteric disturbance; and group D, control Group. Serum β-EP was measured at 10, 20 and 40 minutes after initiation of surgery.

Results: The serum β-EP concentration of group A was 2.74 ± 0.67 ng/ml, 1.57 ± 0.64 ng/ml and 1.64 ± 0.74 ng/ml at 10, 20 and 40 minutes of CO_2 pneumoperitoneum respectively, which was significantly higher than that of the control group ($P < 0.01$). The serum β-EP concentration of group B was 2.53 ± 0.86 ng/ml, 1.46 ± 0.11 ng/ml and 1.34 ± 0.14 ng/ml at 10, 20 and 40 minutes after the 5 cm abdominal incision was made, which was very significantly higher than that of the control group ($P < 0.01$). The serum –EP concentration of group C was 3.77 ± 0.51 ng/ml, 2.99 ± 0.70 ng/ml and 2.67 ± 0.54 ng/ml at 10, 20 and 40 minutes after the 5 cm abdominal incision was made with enteric disturbance. There was a very significant difference in the concentration of serum β-EP at 10, 20 and 40 minutes between Group C and Group B ($P < 0.01$).

Conclusion: Both enteric disturbance and CO_2 pneumoperitoneum are important stimulating factors inducing stress responses in rats. Enteric disturbance may accentuate the severity of stress responses in laparotomy.

Keywords: β-endorphin, pneumoperitoneum, laparoscopy, stress responses.

INTRODUCTION

Carbon dioxide (CO_2) pneumoperitoneum, abdominal incision and especially enteric disturbance are very important stressful stimuli of abdominal surgery including laparoscopy. Surgery is a stressful stimulus that elicits inflammatory, endocrine and metabolic responses as represented by increased levels of stress hormones, leading to substrate mobilization. These changes together constitute the stress response. The stress response caused by surgery is conditioned by several factors such as anxiety, incision size, enteric disturbance (enteric exposure and drawing), exposure of abdominal organs to air, temperature change, operation duration, pain, hemorrhage, and infection. To obtain excellent exposure in laparotomy, it is necessary to incise the abdomen and pull the intestine. Avoidance of enteric disturbance, less hemorrhage, smaller incisional size and shorter operation duration contribute to lowering stress responses in laparoscopic surgery. Laparoscopic surgery and induction of pneumoperitoneum cause minimal activation of stress hormones, resulting in a lower stress response on the part of the patient, and possibly a shorter recovery time.

Serum β-endorphin (β-EP) and cortisol levels are often elevated in animals and humans under major stress conditions including perioperative procedures. Increased generation of β-EP and cortisol contributes to trauma-related acute phase reaction and hypermetabolic response. Secretion of β-EP and cortisol plays a central role in mediating metabolic responses to stress, and there is a linear correlation between cortisol values and the severity of injury. As β-EP is positively correlated with cortisol, and both hormones are the result of corticotropin-releasing hormone (CRH) activation, increased circulating β-EP seems to be part of the response to stress.

In this experimental study, the effect of enteric disturbance and carbon dioxide (CO_2) pneumoperitoneum on stress response was studied in a rat model. Serum β-EP of SPF-grade male SD rats was measured at different time points after initiation of surgery under the experimental condition of CO_2 pneumoperitoneum at 1.0 kPa, a 5 cm abdominal incision without enteric distrubance, or a 5 cm abdominal incision with enteric disturbance to see whether intestinal disturbance as an important factor increased the degree of stress response, and whether reducing intraoperative traction on the intestine reduced the extent and shortened the duration of perioperative stress response, thus reducing trauma and promoting patient rehabilitation.

MATERIALS AND METHODS

Animal Grouping

SPF-grade SD male rats weighing 190-220 gm (Shanghai Laboratory Animal Center, Chinese Academy of Sciences) were given free access to tap water and pelleted food throughout the course of study. Laboratory temperature was maintained at 23ºC and relative humidity at 48%.

Grouping: 120 rats were equally randomized to four groups before intraperitoneal anesthesia with 1% sodium thiopental (0.5 ml/100 gm). In group A, CO_2 pneumoperitoneum was established by abdominal paracentesis at a stable pressure of 1.0 kPa using a pneumoperitoneum machine (STORZ 26020S); in group B, a 5 cm abdominal incision was made on the upper abdomen of rats without any disturbance on the incision and abdominal organs; in group C, a 5 cm abdominal incision was made on the upper abdomen of rats with pulling the intestine with a retractor using 5N pulling power; and in group D, only intraperitoneal anesthesia was performed without any surgical procedure. Serum β-EP was measured at 10, 20 and 40 min postoperatively in every other 10 rats of each group. Serum β-EP was measured in another 10 rats immediately after i.p. anesthesia as normal level.

Estimation of Serum β-EP Levels

Reagents and equipment included a plasma β-EP Kit (Department of Neurobiology of the Second Military Medical University, Shanghai, China), a pneumoperitoneum machine (STORZ 26020S), a refrigerated centrifuge (DL-8R, Centrifugal Machinery Research Institute, Shanghai), and RIA measuring instrument (SN-695, Shanghai).

2 ml blood samples drawn by cardiac puncture were collected in heparinized Vacutainer tubes containing pre-cooled 0.3 mol/LEDTA-2Na (20 mg/l) and aprotinin (500 U/ml), and centrifuged immediately at 3000 gm for 15 minutes. The plasma was stored at 75°C for estimation of the serum β-EP concentration by using a commercial radioimmunoassay kit.

Statistical Analysis

Significant interactions were decomposed by using simple main effects F tests. The significance was evaluated at a level of 0.05. All statistical analyses were performed with the SPSS statistical software package, version 10.1, by a personal computer.

RESULTS

CO_2 Pneumoperitoneum Increases Serum β-EP. The mean serum β-EP concentration was 0.61 ± 0.35 ng/ml immediately after IP anesthesia in the control group. The plasma β-endorphin concentration increased after the establishment of the CO_2 pneumoperitoneum gradually, and rose to the peak at 10 minutes after continuum of the CO_2 pneumoperitoneum, and then decreased at 20n and 40 minutes gradually. The serum β-EP concentration in group A was 2.74 ± 0.67 ng/ml, 1.57 ± 0.64 ng/ml and 1.64 ± 0.74 ng/ml at 10, 20 and 40 minutes respectively, *vs* 0.61 ng/ml, 0.65 ng/ml and 0.64 ng/ml in the control group ($P < 0.01$).

Incising the Abdomen Increases Plasma β-EP

The serum β-EP concentration increased gradually after the 5 cm incision was made on the abdomen, rose to the peak at 10 minutes, and then decreased at 20 and 40 minutes gradually. The serum β-EP concentration of group A was 2.87 ± 0.47 ng/ml, 1.58 ± 0.61 ng/ml and 1.41 ± 0.79 ng/ml at 10, 20 and 40 minutes, respectively, which was significantly higher than that of the control group ($P < 0.01$).

Enteric Disturbance Accentuates the Severity of Stress Response

In group A, B and C, serum β-EP was significantly increased at 10, 20 and 40 minutes compared with the control ($P < 0.01$). There was no significant difference in plasma β-EP at 10, 20 and 40 minutes between group A and B, but the

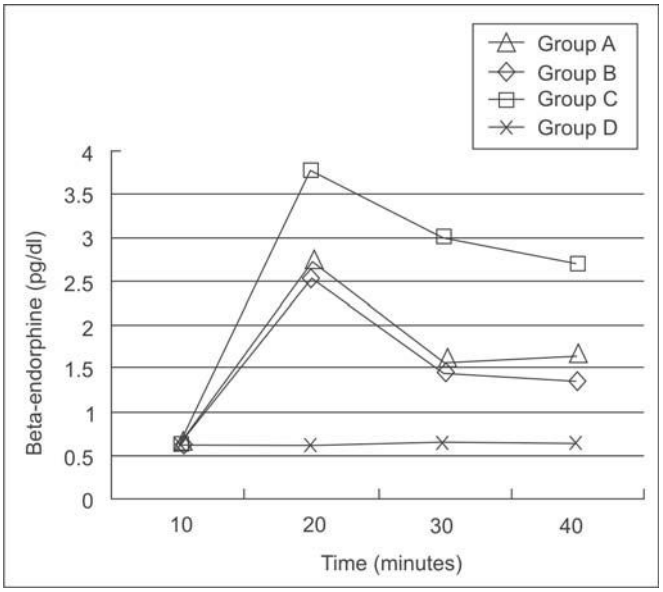

Fig. 1: Serum β-EP was significantly increased at 10, 20 and 40 minutes in group A, B and C as compared with that of the control ($P < 0.01$). There was significant difference in plasma β-EP at 10, 20 and 40 minutes between group B and C ($P < 0.01$), and also between Group A and C ($P < 0.01$), but there was no significant difference between group A and B ($P > 0.05$)

difference between group A and C was significant ($P < 0.01$). It was 3.77 ± 0.51 ng/ml, 2.99 ± 0.70 ng/ml and 2.67 ± 0.54 ng/ml at 10, 20 and 40 minutes respectively in group C, *vs* 2.53 ± 0.86 ng/ml, 1.46 ± 0.11 ng/ml and 1.34 ± 0.14 ng/ml in group B ($P < 0.01$).

DISCUSSION

Over the past few decades, corticotropin-releasing factor (CRF) signaling pathways have been shown to be the main coordinators of endocrine, behavioral and immune responses to stress.[1-4] The central effectors of stress response are the corticotrophin-releasing hormone (CRH) and locus coeruleus-norepinephrine (LC-NE) /sympathetic systems. The CRH system activates stress response and is subject to modulation by cytokines, hormones and neurotransmitters. This stress system is tonically active, but both physical and emotional stressors that exceed a critical threshold increase its activity further. The principal role of glucocorticoids during the stress response is thought to be restraint of the effectors of stress response.[5,6]

β-EP is an opioid peptide representing the C-terminal 31 acid residue fragment of proopiomelanocortin (POMC). The release of β-EP from the pituitary into the cardiovascular compartment under physical or emotional stress has been frequently reported. It is well-established that in the pituitary gland CRH stimulates the release of beta-endorphin via a cAMP-linked mechanism.[7-9] Guillemin et al first reported that β-EP was released from the pituitary into the blood in rats under stress, and that all kinds of stress could stimulate the secretion of serum β-EP, which was controlled by the hypothalamus, mainly by adenohypophysis synthesis, and from POMC and its precursor substances.[10,11] The concentration of serum β-EP increased with stress responses caused by different factors, reached the peak 5 ~ 10 minutes after continuum of the stress factors, and then decreased gradually.

In parallel with an increase in plasma β-EP concentration during stress, an elevation in adrenocorticotropic hormone (ACTH) and cortisol plasma concentrations was observed.[12-14] Stress could stimulate the secretion of serum β-EP, inhibit activity of the sympathetic-adrenal system, regulate the stress intensity, and inhibit secretion of ACTH, glucocorticoid and vasopressin.[15] H. Harbach et al found cortisol as a 'long-term parameter' of the endocrine response to stress.[16-18] In their previous studies, β-endorphin was measured under different stress conditions. In parallel with an increase in β-EP concentrations during stress, an elevation in ACTH and plasma cortisol concentrations was observed. Kho and colleagues measured a significant increase in β-EP levels during acupuncture and transcutaneous stimulation even before skin incision for abdominal surgery and also before laryngoscopy for intubation had been performed.[19] Elevated serum EP and cortisol levels were observed in animals and humans subjected to major stress. Hamit Okur et al reported that there was a linear correlation between β-EP and cortisol values and the injury severity.[20] Increased β-EP and cortisol generation contributes to the acute phase reaction and hypermetabolic response that accompanies trauma. The secretion of cortisol plays a central role in mediating the metabolic responses to stress. Under perioperative conditions, corticotroph-type POMC derivatives such as ACTH or β-EP immunoreactive material (β-endorphin IRM) have been reported to be released in conditions of preoperative stress, surgical injury, or postoperative pain.[21,22] Study of Marschall, et al indicates that although β-END and ACTH are both produced by the pituitary and derived from a common precursor, the type of stimuli (pre-*vs* postsurgical stress) seems to differentially affect their plasma levels.[23]

The stress response caused by surgery is conditioned by several factors such as anxiety, incision size, enteric disturbance (enteric exposure and drawing), exposure of abdominal organs to air, temperature change, operation duration, operated organ and operative type, pain, hemorrhage and infection. Avoidance of enteric disturbance, less hemorrhage, smaller incision size and shorter operation duration contribute to lowering stress response of laparoscopic surgery. To obtain excellent exposure in laparotomy, it is necessary to incise the abdomen and pull the intestine. As laparoscopic surgery and induction of pneumoperitoneum cause minimal activation of the stress hormones, they should result in a lower stress response on the part of the patient, and possibly a shorter recovery time. But whether reducing intraoperative pulling of the intestine truly reduced the extent and shortened the duration of perioperative stress response, thus reducing trauma and promoting patient rehabilitation was not conclusive.

The results of this study show that serum β-EP was elevated, and the degree of elevation was related to the severity of injury. Surgery is a stressful stimulus that elicits inflammatory, endocrine and metabolic responses consisting of increased levels of stress hormones, leading to substrate mobilization.[24,25] These changes together constitute the stress response. Laparoscopic surgery causes minimal activation of the stress hormones while laparotomy results in a more obvious response of the stress hormones, probably due to

increased tissue trauma and less enteric disturbance in laparoscopic surgery.[26,27] β-EP levels increased with pain increasing. Since β-EP was positively correlated with cortisol, and both hormones are the result of CRH activation, increased circulating β-EP seemed to be part of the response to pain and/or stress.[28]

Perioperative serum β-EP concentration change of laparoscopic laparoscopic cholecystectomy (LC) and open cholecystectomy (OC) is very important, and the plasma β-EP concentration is in parallel with the extent and duration of trauma.[14] Intestinal disturbance such as intestinal stretch, exposure of abdominal organs to air or temperature change is part of surgical trauma. Without disturbance to the gastrointestinal tract and exposure of abdominal organs to air may be the important mechanism of mini-invasive surgery, such as LC. CO_2 pneumoperitoneum and intestinal disturbance caused the stress response in rats, and induced the central nervous system to stimulate the hypothalamus releasing CRH, which stimulates the pituitary gland to secrete β-EP. β-EP in group A (CO_2 pneumoperitoneum at 1.0 kPa) or Group B (5 cm abdominal incision without enteric disturbance) increased significantly, indicating that these two experimental conditions can cause stress response in rats, though the difference between the two groups was insignificant ($P > 0.05$). There was a very significant difference in plasma β-EP at 10 and 20 minutes and a significant difference at 40 minutes between group C and B, suggesting that intestinal disturbance can increase the extent of the stress response under the experimental conditions. This experimental study also explored the relationship between intestinal disturbance and serum β-EP in rats. Reducing intraoperative traction on the intestine can reduce the extent and shorten the duration of perioperative stress response, thus reducing trauma and promoting patient rehabilitation.[29]

CONCLUSION

Our results suggest that intestinal disturbance as an important factor can increase the degree of stress response, and may therefore be an important mechanism for minimally invasive intervention such as LC without interference from intestinal traction.

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