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Effect of Morphine and Lacosamide on Levels of Dopamine and 5-HIAA in Brain Regions of Rats with Induced Hypoglycemia

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Abstract: The study aimed to determine the effect of morphine and lacosamide on levels of dopamine and 5-HIAA in a hypoglycemic model. Female Wistar rats (n = 30), mean weight of 180 g were treated as follow: Group 1 (control) received 0.9% NaCl, Group II; morphine (10 mg kg^{-1}), Group III; lacosamide (10 mg kg^{-1}), Group IV; insulin (10 U.I. per rat), Group V; morphine (10 mg kg^{-1})+insulin, Group VI; lacosamide (10 mg kg^{-1})+insulin. All administrations were made intraperitoneally every 24 h, for 5 days. Animals were sacrificed after the last dose to measure the levels of glucose in blood; dopamine and 5-HIAA in cortex, hemispheres and cerebellum/medulla oblongata regions. Levels of glucose decreased significantly in animals treated with morphine, lacosamide and all groups that received insulin alone or combined with respect to control group. Levels of Dopamine diminished significantly in cortex and increased significantly in hemispheres of animals that received morphine. In cortex, 5-HIAA increase significantly in the groups treated with morphine, morphine+insulin and lacosamide+insulin, however a significant decrease of the same substance was witnessed in cerebellum and medulla oblongata of animals that received morphine or lacosamide plus insulin. GSH increased significantly in cortex and cerebellum/medulla oblongata of animals treated with morphine and lacosamide alone or combined with insulin. Lipid peroxidation decreased significantly in cortex and cerebellum/medulla oblongata of groups that received lacosamide alone or combined with insulin. These results indicate that hypoglycemia induced changes in cellular regulation while morphine and lacosamide are accompanied by biochemical responses.

Key words: Hypoglycemia, opioids, biogenic amines, glutathione, morphine

INTRODUCTION

The opioid analgesics, commonly exemplified by morphine, represent the best option for the treatment of severe pain and for the management of chronic pains. Although, this group of analgesics are not considered first line treatments in neuropathic pain, they continued to be the most consistent and effective class of drugs for this condition. Opioids should be applied to the right individuals with strict medical supervision (Bruera *et al*., 2004). It is a known fact that constant and long use of opioids leads to the need of more and more doses to maintain the same effect of pain relief (Ossipov *et al*., 2005). This point constitutes the major problems associated with chronic use of morphine, but the knowledge of the molecular mechanisms involved in this tolerance is not elucidated yet. There is an inverse relationship between diabetic hypoglycemia and the potency of morphine (Simon and Dewey, 1981). This occurs because opiates inhibit insulin signaling through direct between the downstream signaling pathways of μ-opioid receptor MOR and insulin receptor (Li *et al*., 2003). Diabetically induced hypoglycemia occurs as a result of inadequate insulin therapy which is one of the principal factors that could cause dysfunctions of dopaminergic and serotonergic systems of the brain (Shpakov, 2012), as well as neuronal death or cognitive impairment (Won *et al*., 2012).

Neuropathic pain has been associated with diabetes and it is often refractory to conventional therapies, however, the validation of novel analgesics such as lacosamide (Doty *et al*., 2007) is needed. The use of lacosamide as monotherapeutic drug for diabetic neuropathic pain is still under research.

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Several studies on the activity of enzymes, including Glutathione (GSH), have demonstrated changes in their behaviors (Kerimov, 2004). Glutathione is the principal regulator of redox equilibrium that protects the tissues against the actions of free radicals, especially those acting on cell membrane (Beckman *et al*., 1990). Oxidative damage in early ages of development, when metabolism and growth are at peak, depends on the presence of GSH and CNS is highly sensible to it (Ebadi *et al*., 2007). Depletion of GSH in peripheral organs after acute systemic and central opioid administration has been found in previous studies (Goudas *et al*., 1997). The principal receptor in narcotic addiction is the μ-opioid receptor. This receptor plays an important role in addiction, gene regulation and synaptic remodeling (Sadee *et al*., 2005). MOR is N-glycosylated protein involved in the regulation of G proteins. It is a member seven transmembrane G-protein-coupled receptors (Garzon *et al*., 2005). In nervous tissues, the G proteins are always found as dimers, indicating that this association is required for their function between MOR and G subunits for the activation of antinociceptive effects.

On the other hand, studies with morphine suggest that NO may play an important role in the development of tolerance to opiate (Lue *et al*., 1999). This could explain why there was an increase of NO in brain of mice with dependence of morphine. This increase was counteracted by the administration of melatonin, an important metabolite of serotonine (5-HT) capable of capturing free radicals in damaged tissue (Ebadi *et al*., 2007) and of induction of an increase in 5-Hydroxyindole-3-acetic Acid (5-HIAA) levels (Hirose, 1992). Moreover, in the presence of morphine, it can increase oxidative metabolism (Enrico *et al*., 1998). Thus, the aim of the present study was to determine the effect of morphine sulfate administration and lacosamide on the degree of oxidative stress in hypoglycemic brain of rat measured by the brain levels of GSH, lipid peroxidation, 5-HIAA and Dopamine using spectrophotometric and fluorimetric methods.

MATERIALS AND METHODS

Thirty female Wistar rats of 7 weeks of age and a mean weight of 180 g were employed in the study. The animals were divided into 6 groups of 5 rats each and were kept in closed boxes all through the study under a light: dark period of 12:12. The administration of treatments was as follows: Group 1 (control) received 0-9% Na Cl, Group II; morphine sulfate (10 mg kg^{-1}), Group III; lacosamide (10 mg kg^{-1}), Group IV; insulin (10U.I per rat), Group V; morphine sulfate (10 mg kg^{-1})+insulin 10 U.I, Group VI; lacosamide (10 mg kg^{-1})+insulin 10 U.I. All drug administration consists of a single intraperitoneal dose every 24 h, during 5 days. Animals were sacrificed by decapitation 60 minutes after receiving the last dose of drug and their brains were immediately placed in 0.9% cold NaCl. The brains were divided into cortex, hemispheres, cerebellum/medulla oblongata and then homogenized in 0.05M Phosphate Buffer Solution (PBS), pH 7.4, for determination of Dopamine, 5-HIAA, lipid peroxidation and GSH. All samples were frozen at -20°C and stored until analyzed. The study was carried out with complete and strict compliance to National and International Rules for Animal Care.

Measurement of Glucose: The procedure to measure blood glucose was carried out in all groups of animals at the moment of sacrifice. Ten micro liter of non-anticoagulant fresh blood were obtained and smeared on a reactive filter paper in Accu-Chek active (Roche Mannheim Germany) equipment and the concentration was read in mg dL^{-1}.

Dopamine (DA) measurement: DA levels were measured in the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), using a modified version of the technique reported by Guyon *et al*. (1984). An aliquot of the $HClO_4$ supernatant and 1.9 mL of buffer (0.003 M octyl-sulphate, 0.035 M KH_2PO_4, 0.03 M citric acid, 0.001 M ascorbid acid), were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 282 nm excitation and 315 nm emission lengths. The FL Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nmol g^{-1} of wet tissue.

5-Hydroxyindole-3-acetic acid (5-HIAA) measurement: 5-HIAA levels were measured in the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), with a modified version of the technique reported by Beck *et al*. (1977). An aliquot of the $HClO_4$ supernatant and 1.9 mL of acetate buffer 0.01 M pH 5.5 were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 296 nm excitation and 333 nm emission lengths. The FL

Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nmole g^{-1} of wet tissue.

Glutathione (GSH) measurement: GSH levels were measured in the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9,000 rpm for 10 min (Hettich Zentrifugen, model Mikro 12-42, Germany), with a modified version of the technique reported by Hissin and Hilf (1976). An aliquot of the $HClO_4$ supernatant and 1.8 mL of phosphate buffer pH 8.0, with 0.2 % EDTA and 100 μL of ortho-phthaldialdehyde (OPA) in 1 mg mL^{-1} in methanol were placed in a test tube. The mixture was incubated for 15 min at room temperature in total darkness and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 350 nm excitation and 420 nm emission lengths. The FL Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nmoles g^{-1} of wet tissue.

Lipid peroxidation (TBARS) measurement: TBARS determination was carried out using the modified technique of Gutteridge and Halliwell (1990) as described below: From the homogenized brain in tris-HCl 0.05 M pH 7.4, 1 mL was taken and to it was added 2 mL^{-1} of thiobarbaturic acid (TBA) containing 1.25 g of TBA, 40 g of trichloroacetic acid (TCA) and 6.25 mL^{-1} of concentrated chlorhydric acid (HCL) diluted in 250 mL^{-1} of deionized H_2O. The mixture was heated to boiling point for 30 min (Thermomi×1420). The samples were later put in ice bath for 5 min and were centrifuged at 700 g for 15 min (Sorvall RC-5B Dupont). The absorbance of the floating tissues was read in triplicate at 532 nm in a spectrophotometer (Heλios-α de UNICAM). The concentration of reactive substances to the thiobarbaturic acid (TBARS) was expressed in μM of Malondialdehyde/g of wet tissue.

Statistical analysis: The results were analyzed by two-way ANOVA and Tukey test. Contrasts and comparisons for all pairs were obtained by Dunnett's and Kramer HSD statistical tests, respectively. Values of $p<0.05$ were considered statistically significant (Castilla, 2011). The JMP version 8.0.0 was used.

RESULTS

Table 1 shows the concentration levels of blood glucose of rats treated with morphine and lacosamide in the presence of insulin. This concentration decreased significantly ($p = 0.005$) in the groups treated with morphine, lacosamide and insulin alone or combined with respect to the control group.

Dopamine levels in brain regions of animals treated with morphine and lacosamide in the absence or presence of insulin diminished significantly ($p = 0.0098$) in cortex of animals that received morphine and increased significantly ($p = 0.0087$) in hemispheres region when compared with the control group (Table 2).

Table 1: Glucose levels in blood of hypoglycemic rats treated with morphine and lacosamide. Mean±SD

Groups	Glucose (mg dL^{-1})
Control (vehicle)	130.0±6.2*
Morphine	119.4±4.0
Lacosamide	116.4±7.1*
Control+insulin	79.4±36
Morphine+insulin	54.4±10
Lacosamide+insulin	68.8±37

Values are Mean±SD (n = 5): Kruskall-Wallis *p<0.05: Control vs lacosamide, morphine, morphine+insulin. Lacosamide vs lacosamide+insulin

Table 2: Concentration of dopamine, 5-HIAA, GSH and TBARS in brain regions of hypoglycemic rats treated with morphine and lacosamide

	Dopamine (nM)			5-HIAA (nM)			GSH (nM)			TBARS(μMmalondialdehyde)		
Groups	C	H	CM	C	H	CM	C	H	CM	C	H	CM
Control (vehicle) (5)	742.24	891.16	506.06	306.33	489.51	400.17	423.2*	555.20	443.7*	3.69*	4.36	4.85*
Morphine (5)	773.84*	967.42*	612.74	351.65*	510.22	378.25	415.02	553.80	533.06	2.16	3.02	5.66
Lacosamide (5)	910.28	1194.04	514.35	445.71	569.57	364.79*	454.55*	562.30	521.5*	2.58	5.11	6.17
Control+insulin (5)	655.17	981.75	551.49	318.27*	501.65	368.24*	487.02	563.70	477.10	2.29*	4.46	7.98*
Morphine+insulin (5)	656.54	925.12	817.37	385.27	493.00	259.04	568.96	562.10	381.30	2.05	4.90	3.83
Lacosamide+insulin (5)	713.94	797.53	511.13	375.57	475.93	302.75	580.62	506.40	418.80	4.47	4.14	5.19

C: Cortex, H: Hemispheres, CM: Cerebellum/medulla oblongata; Number of animals. ANOVA Two ways and Kruskal-Wallis *p<0.05. Dopamine: Cortex: p = 0.0098, Morphine vs control, insulin, morphine+insulin. Hemispheres: p = 0.0087. Morphine vs control, lacosamide, insulin. 5-HIAA: Cortex: p<0.001. Control vs morphine, morphine+insulin, lacosamide+insulin. Morphine vs lacosamide, insulin. Insulin vs lacosamide+insulin, morphine+insulin. Cerebellum/Medulla oblongata: p = 0.0186. Control vs lacosamide+insulin, morphine+insulin. Lacosamide vs lacosamide+insulin. Insulin vs lacosamide+insulin. GSH: Cortex: p = 0.0034. Control vs morphine+insulin, lacosamide+insulin. Lacosamide vs morphine+insulin, lacosamide+insulin. Cerebellum/medulla oblongata: p = 0.034. Control vs morphine, lacosamide. Lacosamide vs Lacosamide+insulin. Lipid peroxidation: Cortex: p = 0.0001 Control vs Insulin, lacosamide, lacosamide+insulin. Insulin vs morphine+insulin. Morphine vs morphine+insulin. Cerebellum/medulla oblongata: p = 0.0019. Control vs insulin. Insulin vs lacosamide+insulin, morphine+insulin. Mean values per gram of wet tissue±25% of SD

The levels of 5-hydroxyindole-acetic acid (5-HIAA) increased significantly ($p<0.001$) in animals groups that received morphine or morphine+insulin and lacosamide+insulin and decrease significantly ($p = 0.0186$) in cerebellum/medulla oblongata regions in animals that received morphine or lacosamide plus insulin with respect to the control group (Table 2).

The levels of GSH in cortex and cerebellum/medulla oblongata regions increased significantly ($p = 0.0034$) in animal groups treated with morphine and lacosamide alone or combined with insulin (Table 2).

Lipid peroxidation decreased significantly in cortex ($p = 0.0001$) and cerebellum/medulla oblongata ($p = 0.0019$) regions of animals treated with lacosamide alone or combined with insulin and showed an increase in animals that received morphine plus insulin (Table 2).

DISCUSSION

Currently, the major issue in glycemic control is the neurological management of the patients. Tight glycemic control using intensive insulin therapy is associated with higher rate of hypoglycemia without an improvement in survival rate. For this, adequate nutrition before and during insulin infusion is necessary (Bilotta and Rosa, 2012). In the present study that recommendation was considered and therefore was a slight decrease of glucose on blood that confirmed the hypoglycemia model postulated by Bilotta and Rosa (2012).

In this study, dopamine levels decreased significantly in cortex and increased in hemisphere of all animals that received morphine. It is known that morphine aggravates neurotoxic effects of hypoxic/hypoglycemic model and delays their onset by 1 to 3 h (Ammon-Treiber *et al.*, 2005).

The characteristic increase and reduction of 5-HIAA levels in cortex and cerebellum/medulla oblongata respectively under morphine treatment could be alluded to the high serotonergic activity and the maturity of the brain (Fernstrom and Wurtman, 1972; Hernandez, 1973). Some studies (Enrico *et al.*, 1998), suggests that morphine increases serotonin metabolism and gives rise to an increase in its metabolites which are important compounds in the reduction of oxidative damage in brain. With regard to glutathione levels which witnessed an increase in hypoglycemic animals suggesting that morphine and lacosamide play an important role in protecting the brain from oxidative stress. This observation was confirmed in the study of Goudas *et al.* (1999), who found acute decreases in glutathione levels in samples of cerebrospinal fluid obtained from cancer patients after intracerebroventricular doses of morphine for intractable cancer pain. Such doses could deplete glutathione, a powerful antioxidant, thereby rendering the central nervous system vulnerable to damage from oxidative stress. It is suggest that this oxidative stress is induced by NO production which is synthesized in selected neuron populations during the opiate syndrome that alters GSH metabolism in brain (Jhamandas *et al.*, 1996).

Likewise, age (Driver *et al.*, 2000), gender (Peckham *et al.*, 2005) and genetic variability (Klepstad *et al.*, 2005) are determining factors that define the effect of morphine, owing to variations in μ-opiate receptors and protein G.

Lipoperoxidation decreased as a consequence of lacosamide treatment but increased with morphine plus insulin treatment. This result is in accordance with the results of previous studies done in our laboratory where a similar evidence was obtained in young and adult animals treated with acute doses of morphine sulfate (Guzman *et al.*, 2006), culminating to increased oxidative lipoic damages.

CONCLUSION

The results of the study suggest that hypoglycemia induces changes in cellular regulation while morphine and lacosamide induce biochemical responses. Effect of oxidative stress may be involved in these responses.

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