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Morphine Alters Calcium Levels and ATPase Activity in the Brain Regions of Rats with Insulin-induced Hypoglycemia

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ABSTRACT

The objective of this study was to determine if morphine and lacosamide alter ATPase activity or calcium levels in the brain of rats with insulin-induced hypoglycemia. Wistar rats received insulin and morphine or lacosamide (both at 10 mg kg^{-1}) for 5 consecutive days. Blood glucose was measured and after sacrifice, their brains were extracted to measure ATPase activity and calcium levels in cortex, hemispheres and cerebellum/medulla oblongata regions. Glucose decreased significantly in animals treated with morphine and lacosamide. ATPase activity decreased significantly in hemispheres of animals received insulin alone or combined with lacosamide or morphine, but increased in cerebellum/medulla oblongata region. Calcium increased in hemispheres and cerebellum/medulla oblongata of animals with morphine and insulin alone. The V_{max} decreased significantly in hemispheres of animals with morphine and lacosamide in the presence of insulin. The results indicated that morphine and lacosamide alter ATPase activity and calcium levels in rat's brain with insulin-induced hypoglycemia.

Key words: ATPase, brain, calcium, hypoglycemia, morphine

INTRODUCTION

Opioid analgesics, commonly exemplified by morphine, represent the best option for the treatment of severe pain and for the management of chronic pain. Although this group of drugs is not considered as first line treatments in neuropathic pain, but still, they remain the most consistently effective class of substances for this condition. Opioids must be used only in appropriate individuals and under close medical supervision (Bruera *et al*., 2004). Besides, it is well recognized that prolonged use of opioids is associated with a requirement for ever-increasing doses in order to maintain pain relief at an acceptable and consistent level (Ossipov *et al*., 2005), however, the major problems associated with tolerance of the chronic use of morphine is still unclear.

The potency of morphine is inversely correlated with diabetic-induced hypoglycemia (Simon and Dewey, 1981), because opiates can inhibit insulin signaling through direct cross talk between downstream signaling pathways of μ-opioid receptor (MOR) and insulin receptor (Li *et al*., 2003). Diabetic-induced hypoglycemia occurs as a result of inadequate insulin therapy which is the main factor that brings about biochemical dysfunctions of the brain (Shpakov, 2012) and neuronal death or cognitive impairment (Won *et al*., 2012). Neuropathic pain has been associated with diabetic disease. This pain is often refractory to conventional pharmacotherapies and therefore, requires

novel analgesics as lacosamide (Doty *et al*., 2007), a drug which is a new chemical entity employed in the treatment of epilepsy and which is still under investigation as monotherapy for diabetic neuropathic pain.

Some neurological disorders have been implicated in deregulation of Ca^{2+} homeostasis and high-affinity Ca^{2+} transport ATPase plays a crucial role in controlling cytosolic Ca^{2+} (Berrocal *et al*., 2009). Besides, studies on activity of enzymes have demonstrated changes in their behavior, including ATPase enzyme, whose enzymatic activities have been implicated in neurotoxicity and are possibly related to the pathogenesis of epilepsy and neurodegenerative disorders (Conto and Venditti, 2012). During morphine tolerance, adaptive cellular changes take place in cerebral Na^+, K^+-ATPase activity and these are functionally relevant for morphine-induced antinociception (Gonzalez *et al*., 2012). Morphine indirectly enhances Na^+, K^+ATPase activity in brain by activating μ-opioid receptors and G(i/o) proteins (Masocha *et al*., 2002).

The μ-opioid receptors (MOR) are involved in narcotic addiction. These receptors potentially play a key role in addiction as well as in gene regulation and synaptic remodeling (Sadee *et al*., 2005). The MOR which belongs to the family of seven-transmembrane G-protein-coupled receptors, is a heavily N-glycosylated protein that regulates G proteins (Garzon *et al*., 2005). In nervous tissues, G proteins are always found as dimmers, indicating that this association is required for their function between MOR and G subunits for activation of antinociceptive effects.

Thus, the aim of the present study was to determine the effect of morphine sulfate administration and lacosamide on ATPase activity and calcium levels in hypoglycemic rat brain model.

MATERIAL AND METHODS

Thirty-six Wistar rats, 7 weeks of age and 180 g mean weight, were used in the study. The rats were kept under a light:dark period of 12:12 in closed boxes and were divided in six groups of 6 rats each and were made to receive the following treatments: Group 1: 0.9% NaCl (control), Group 2: Morphine sulfate (10 mg kg^{-1}), Group 3: Lacosamide (10 mg kg^{-1}), Group 4: Insulin (10 UI), Group 5: Morphine sulfate (10 mg kg^{-1})+insulin (10 UI) and Group 6: Lacosamide (10 mg kg^{-1})+insulin (10 UI). All animals received their respective treatments intraperitoneally every 24 h during 5 days. About 60 min later, animals were sacrificed by decapitation and their brains were immediately placed in 0.9% cold NaCl. The brain regions were separated in cortex, hemispheres, cerebellum/medulla oblongata and then homogenized in 0.05 M Phosphate Buffer Solution (PBS), pH 7.4, for determination of ATPase activity and calcium. All samples were frozen at -20°C until analyzed. The study was carried out in accordance with National and International rules for animal care.

The procedure to measure blood glucose was carried out in all groups of animals at the moment of sacrifice. The 10 μL 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}.

Measurement of calcium: The procedure to measure calcium was performed using supernatant liquid from the brain homogenate from each tissue region of all animal groups, using Ca-Color Arsenazo III AA direct colorimetric method kit (Wiener Lab Rosario, Argentina). The concentration was obtained using an internal standard and was reported in mg g^{-1} wet tissue.

Procedure to measure ATPase activity: The activity of ATPase was assayed using the method proposed by Calderon-Guzman *et al*. (2005). One milligram (10%) w/v of homogenised brain tissue

in 0.05 M tris-HCl at pH 7.4 was incubated for 15 min in a solution containing 3 mM $MgCl_2$, 7 mM KCl and 100 mM NaCl. To this was added 0.5, 1, 2, 3 and 4 mM tris-ATP and incubated for another 30 min at 37°C in a shaking water bath (Dubnoff Labconco). About 100 μL 10% trichloroacetic acid w/v was used to stop the reaction and samples were centrifuged at 100 g for 5 min at 4°C. Inorganic phosphate (Pi) was measured in duplicates using one supernatant aliquot as proposed by Fiske and Subbarow (1925). Supernatant absorbance was read at 660 nm in a Helios-α, UNICAM spectrophotometer and expressed as μM Pi g^{-1} wet tissue per minute.

Statistical analysis: The results were analyzed by two-way ANOVA, Student t-test and Kruskal-Wallis test. Contrasts and comparisons for all pairs were obtained by Dunnett and Kramer HSD statistical tests, respectively. Values of $p<0.05$ were considered statistically significant (Castilla-Serna and Cravioto, 1999). The JMP version 10.0 for academic was used.

RESULTS

Table 1 shows the blood glucose levels in all the groups of rats. This parameter was found to decrease significantly ($p<0.005$) in the group of rats that were treated with morphine and lacosamide as well as in those that received insulin alone or combined with respect to the control group.

ATPase activity in brain regions of hypoglycemic rats treated with morphine and lacosamide decreased significantly ($p<0.05$) in hemisphere regions of the groups of rats that received insulin alone and combined with lacosamide and morphine, but increased in cerebellum/medulla oblongata region when compared with the control group (Fig. 1).

Values of ATPase activity in rat brain regions is presented in Table 2. The V_{max} of animals treated with morphine and lacosamide in the presence of insulin decreased significantly

Table 1: Glucose levels in blood of hypoglycemic rats treated with morphine and lacosamide

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

Results are taken as Mean±SD, *n = 6 animals per group. Kruskall-Wallis **p<0.05, Control vs. lacosamide, morphine, morphine+insulin, Lacosamide vs. lacosamide+insulin

Table 2: ATPase activity in brain regions of hypoglycemic rats treated with morphine and lacosamide

	Cortex		Hemispheres		Cerebellum/medulla oblongata	
Groups*	Km	V_{max}	Km	V_{max}	Km	V_{max}
Control	1.07±0.87	200.60±76.8	0.82±0.50	241.6±550	0.75±0.35	268.0±56
Lacosamide	1.09±0.60	185.50±40.0	0.60±0.30	231.2±340	1.60±1.30	523.8±505
Morphine	0.70±0.46	160.96±32.0	0.54±0.37	223.0±400	1.13±0.48	319.3±81
Insulin	0.72±0.39	174.70±32.0	1.80±2.40	401.6±295**	0.34±0.25	228.2±32
Insulin+Lacosamide	0.73±0.34	185.40±35.0	0.92±0.50	293.9±490	0.85±0.70	295.2±95
Insulin+Morphine	0.68±0.38	200.00±32.0	1.08±0.70	314.3±860	0.60±0.30	261.0±57

Mean values of Km and Vmax±30% of SD. *n = 6 animals per group, One way ANOVA **p<0.05, Hemispheres: Insulin vs. morphine+insulin, Km = μM g^{-1} wet tissue and V_{max} = μM g^{-1} wet tissue per min

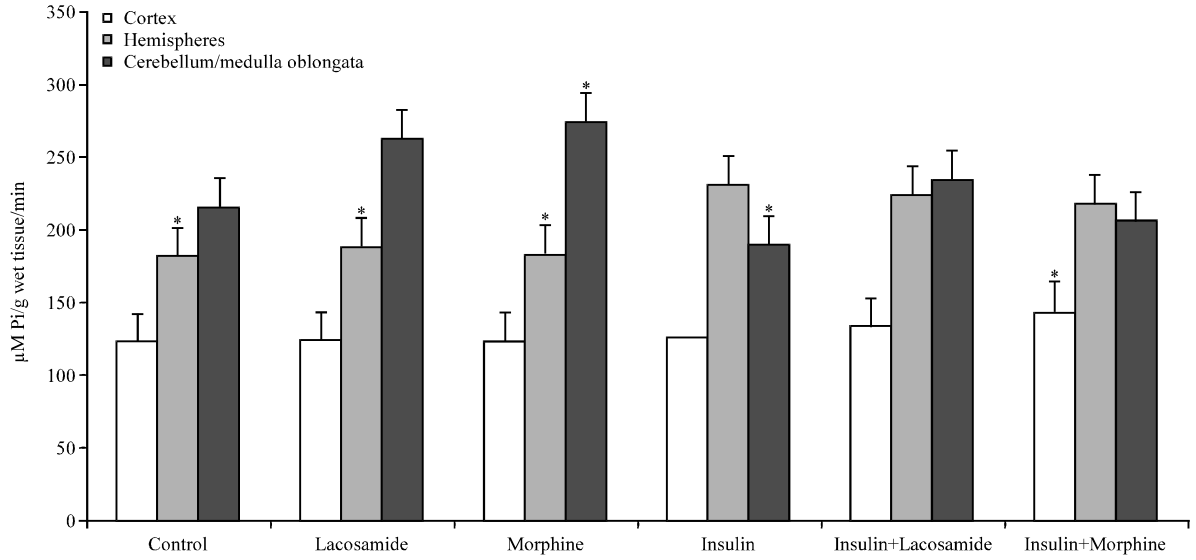

Fig. 1: ATPase activity in brain regions of hypoglycemic rats. Mean values with 4 mM substrate (ATP) ±20% of SD. Kruskal Wallis test *p<0.05

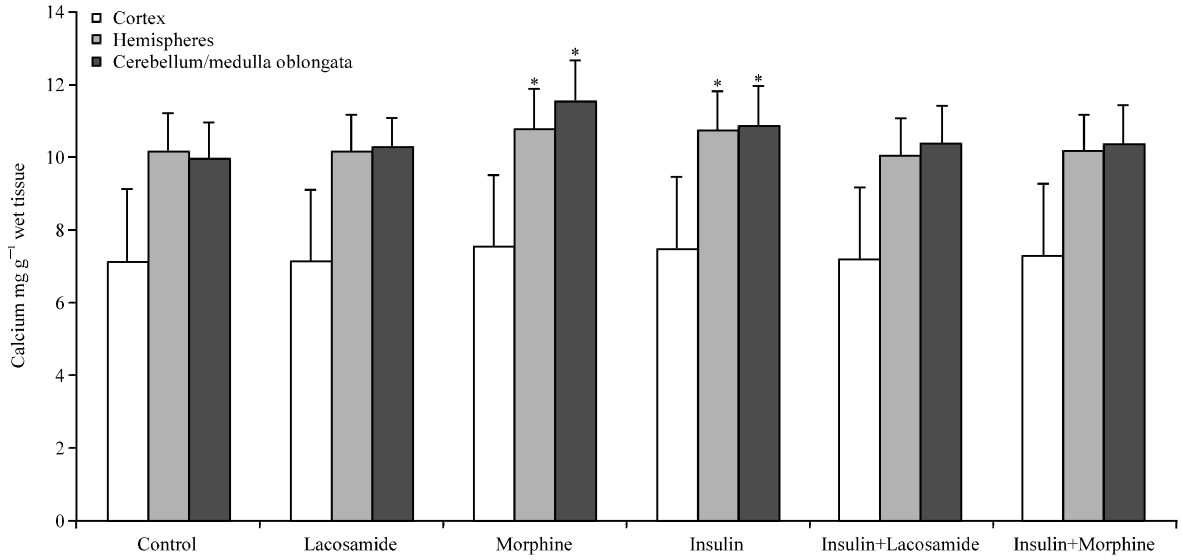

Fig. 2: Calcium levels in brain regions of hypoglycemic rats treated with morphine and lacosamide. Mean values ±10% SD

(p = 0.0048) particularly in hemisphere regions of animals that received insulin plus morphine in comparison with insulin group. Units were: Km = µM g^{-1} wet tissue and V_{max} = µM g^{-1} wet tissue per minute.

Calcium levels increased significantly (p<0.05) in hemispheres and cerebellum/medulla oblongata regions of animals treated with morphine and insulin alone with respect to the control group (Fig. 2).

DISCUSSION

Currently, the major issue in glycemic control for neurocritical care patients is that tight glycemic control using intensive insulin therapy is associated with higher rates of hypoglycemia without an improvement in survival rate. This is reason why some authors recommend adequate nutrition before and during insulin infusion (Bilotta and Rosa, 2012). In the present study, we found a slight decrease in blood glucose level thereby confirming the hypoglycemic model.

The decreasing of ATPase by morphine treatment in hemisphere regions in this study may be due to high density of opiate receptors as was suggested by Pillai and Ross (1986), probably as a consequence of stimulation of protein kinase (PKA) which increased the phosphorylation of Na^+, K^+-ATPase, leading to reduction of enzyme activity (Wu *et al*., 2007). Although, other authors suggest that morphine suppresses Na^+, K^+-ATPase activity by interacting with Fe^{2+} at opioid receptor sites and that this may play a role in suppression of membrane depolarization and release of NA through their stimulatory action on Na^+, K^+-ATPase activity that was probably suppressed by Fe^{2+} in the brain (Nishikawa *et al*., 1990).

With respect to calcium levels, there was a decrease in the concentration as a consequence of morphine treatment and its combination with insulin, suggesting an additive effect of these drugs. Perhaps, the decrease on the activity of Ca^{2+}, Mg^{2+}-ATPase is due to diminished calcium levels (Hoskins *et al*., 1985).

CONCLUSION

In conclusion, the results indicate that morphine and lacosamide alter ATPase activity and calcium levels in rat's brain with insulin-induced hypoglycemia.

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