OPIUM CAN DIFFERENTLY ALTER BLOOD GLUCOSE, SODIUM AND POTASSIUM IN MALE AND FEMALE RATS

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ABSTRACT

To determine the effects of opium on serum glucose, potassium and sodium in male and female Wistar rat, opium solution (60 mg/kg) injected intraperitoneally and the same volume of distilled water was used as control (7 rats in each group). Blood samples were collected at 0, 30, 60, 120, 240 and 360 minutes after injection from orbit cavity and the values of serum glucose, sodium (Na^+) and potassium (K^+) were measured. The data were then analyzed by the repeated measure ANOVA based on sex and case-control group. $P < 0.05$ considered as significant difference. Serum glucose increased significantly at 30, 60, 120 and 240 minutes after opium solution injection, in female rats compared to a control group. However, the male rats had this rise at 30, 60 and 120 minutes after opium solution injection compared to control group. While serum glucose in male rats was significantly higher than females at 30, 60 and 120 minutes, this value was higher in the female rats at 360 minutes. Therefore, serum glucose alterations following opium injection was significantly different in groups and in the sexes at different times.

Sodium (Na^+) rose at 60, 240 and 360 minutes significantly in all rats compared to control group. However, sodium alteration following opium injection was significantly different only between treated and control groups but sex-independent at all times.

Potassium (K^+) increased significantly at 60, 120, 240 and 360 minutes in male rats, compared to a control group. In female rats K^+ significantly raised at 30, 120, 240 and 360 minutes. Therefore, the alteration of K^+ in male and female rats was found time dependent and sex independent.

According to our results, opium increased serum glucose in male and female rats differently, and it interferes with metabolic pathways differently on a gender dependent basis. Opium raised serum Na^+ and K^+, thus it interfere with water regulation and blood pressure via different mechanism.

Key words: Opium, glucose, potassium, sodium, gender.

INTRODUCTION

Opium is a narcotic analgesic drug which is obtained from the unripe seed pods of the opium poppy. To harvest opium, the skin of the ripening pods is scored by a sharp blade. The slashes exude white, milky latex, which dries to a sticky brown resin that is scraped off the pods as raw opium (Opium-Wikipedia, 2006). Opium is used as the raw material for the synthesis of some medications such as morphine, noscapine and papaverine[10%, 6% and 1% of opium respectively] (Hanson, 1995; Gutstien and Akil, 2006). There have been many reports on the effects of morphine, noscapine and papaverine on animals (Venturella, 1995; Molina *et al*., 1994; Ipp *et al*., 1978, 1980; Bossone and Hannon, 1991; Klaff *et al*., 1987; Kamei *et al*., 1998; Hashiguchi *et al*, 1995; Terner *et al*., 2003; Landen *et al*., 2004; Ye *et al*., 1998; Mahmoudian and Mojaverian, 2001; Zacherl *et al*., 2004; Zacherl *et al*., 2003; Beltowski *et al*., 1998; Kraupp and Marz, 1995). It has been reported that morphine increases hormones such as adrenalin, noradrenalin, corticosterone and glucagone (Venturella, 1995; Molina *et al*., 1994; Ipp *et al*., 1978, 1980; Bossone and Hannon, 1991), which affect the metabolism in different ways. Short and long term effects of morphine on the hormone secretion are different (Ipp *et al*., 1978). Noscapine and papaverine are other important component of opium (Gutstien and Akil, 2006). Noscapine is a commonly used antitussive agent in many countries (Landen *et al*., 2004). It binds stoichiometrically to tubulin, affects microtubule assembly and arrests mammalian cells in mitosis, and induces apoptosis (Ye *et al*., 1998). Noscapine has a specific antagonist effect on bradykinin receptors (Mahmoudian and Mojaverian, 2001). Papaverine increases renal blood flow, urine

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output and creatinine clearance (Zacherl *et al*., 2004; Zacherl *et al*., 2003), inhibits phosphodiesterase and reduces Na^+, K^+-ATPase activity (Beltowski *et al*, 1998). It inhibits facilitated transport of nucleobases and sodium dependent active nucleobase transport (Kraupp and Marz, 1995). There are however, more than 20 alkaloids (Venturella, 1995), and more than 70 components (Buchbauuer *et al*, 1994) in opium, thus its effect on metabolism and the endocrine system could therefore be different from pure morphine, noscapine and papaverine. Effect of opium on biochemical parameter in individual human addicts with non-insulin-dependent diabetes mellitus (NIDDM), revealed differences in males and females. Our previous study showed HbA_{1c} and K^+ was higher in diabetic opium addicts (Asadi Karam *et al.*, 2004). However, we could not find any reports on the effects of opium on the blood glucose, K^+ and Na^+ in short and long term. The aim of this study was to show possible roles of opium on these factors in different times in male and female rats.

MATERIALS AND METHODS

Opium was received from the police office. It dissolved in distilled water as 100µg/µl. Wistar rats (14 males and 14 females, 220 to 250 gram body weight) were treated with opium solution (60mg/kg) or distilled water as control (7 rats in each group) through intra peritonealy injection. Blood were collected at 0, 30, 60, 120, 240 and 360 minutes after injection from orbit cavity by thin heparinized tube. Serum glucose was determined using the glucose oxidase method (Barham and Trinder, 1972) (Pars Azemoon Company Tehran, Iran) in a Kone autoanalyzer-specific model, serum sodium and potassium were measured by a Corning flame photometer M480 (Corning limited, United Kingdom).

The data were analyzed using repeated measure ANOVA. The changes of serum glucose, Na^+ and K^+ were determined at 0, 30, 60, 120, 240, 360 minutes, and the alterations of these parameters were analyzed on the basis of sex and case - control group. Harmonity of covariance was measured by Mauchly's test of Sphericity, whereas; F test was used in harmonized covariance in Sphericity Assumed conditions, but in non- harmonized covariance state, Green house – Geisser test was used (table 1). The amount of $P < 0.05$ considered as significant difference.

RESULTS

Serum glucose, as shown in fig. 1, increased significantly at 30, 60, 120 minutes after opium solution injection, then started to decrease gradually at 240 and 360 minutes in male rats compared to control group. Increase of serum glucose at 30, 60, 120, 240 minutes was significant, then this enhancing was decreased at 360 minutes in female rats case group compare to control group. Serum glucose of male rats was higher at 30, 60 and 120 minutes than females significantly, but at 360 minutes, serum glucose of female rats was higher than male rats. Therefore, serum glucose alterations following opium injection was different significantly in groups and in the sexes at different times.

Sodium (Na^+) raised at 60, 240 and 360 minutes significantly in case group's male and female rats compare to control groups (fig. 2). However, sodium alteration following opium injection was different significantly only in groups but the differences were sex-independent at different times.

Potassium (K^+) increased significantly at 60, 120, 240 and 360 minutes in case group male rats, compare to control group. In case group female rats K^+ increased at 30, 120, 240 and 360 minutes significantly but at 60 minutes it decreased compare to control group however it was not significant. Therefore the alteration of K^+ in male and female rats was found time dependent and sex independent (fig. 3).

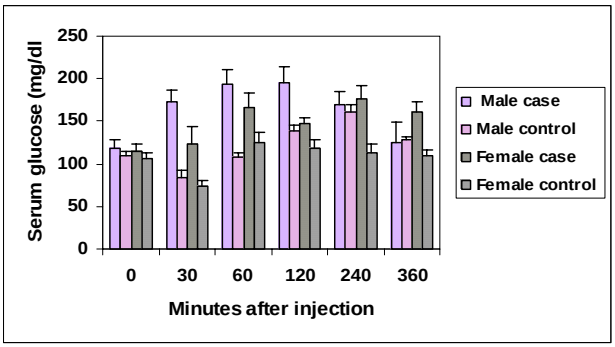

Fig. 1: The concentration of serum glucose in male and female rats given opium solution (60 mg/kg) or distilled water as control. Values are mean ± SEM (n=7 per group).

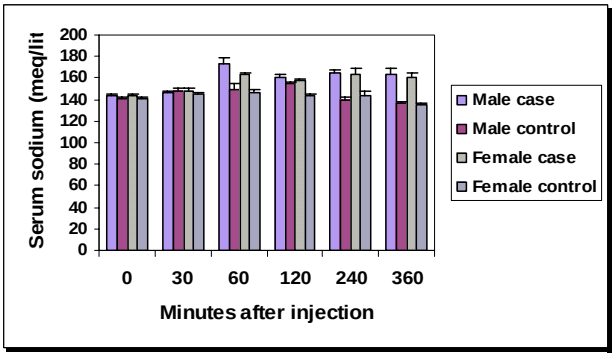

Fig. 2: Serum sodium (Na^+) levels in male and female rats given opium solution (60 mg/kg) or distilled water as control. Values are mean ± SEM (n=7 per group).

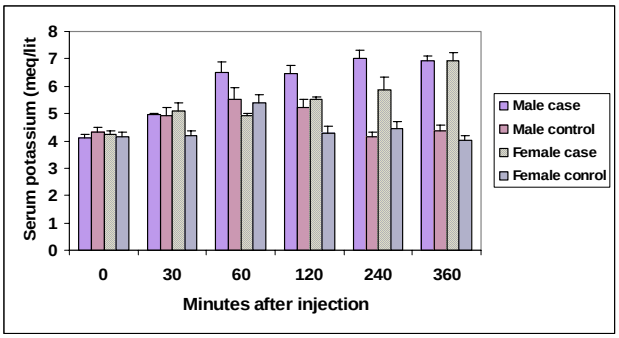

Fig. 3: Serum potassium (K^+) values in male and female rats given opium solution (60 mg/kg) or distilled water as control. Values are mean ± SEM (n=7 per group).

DISCUSSION

The result of the present study showed that increase of serum glucose by opium is time and sex dependent, but only alteration of Na^+ and K^+ were time dependent. Opium has been used as therapeutic medicine for thousand of years. Avicenna the great Iranian physician (980-1037) had prescribed it for cough, anemia and diarrhea (Stephano *et al.*, 2000). Because, opium has been associated with human history and for a long time some people believe that it has therapeutic effect on many disorders. This was the reason why opium prescribed by ordinary people and it make an excuse to explaining to use it. However, addiction has caused severe problems for both the abusers and society. We have previously shown

Table 1: Analysis of serum glucose, sodium (Na^+) and potassium (K^+) according to group (given opium solution) or control (given distilled water) and gender (male or female).

Source		df	F	Sig.
Serum glucose	Sphericity Assumed Greenhouse-Geisser	4 3.226	8.880 8.880	0.000 0.000
Serum glucose* gender	Sphericity Assumed Greenhouse-Geisser	4 3.226	2.496 2.496	.050 0.065
Serum glucose* group	Sphericity Assumed Greenhouse-Geisser	4 3.226	2.735 2.735	.035 .048
Serum glucose * gender * group	Sphericity Assumed Greenhouse-Geisser	4 3.226	5.050 5.050	.001 .003
Error (Serum glucose)	Sphericity Assumed Greenhouse-Geisser	72 58.077		
Serum Na^+	Sphericity Assumed Greenhouse-Geisser	4 2.746	7.813 7.813	.000 .000
Serum Na^+* gender	Sphericity Assumed Greenhouse-Geisser	4 2.746	1.388 1.388	.247 .258
Serum Na^+ * group	Sphericity Assumed Greenhouse-Geisser	4 2.746	10.532 10.532	.000 .000
Na^+* gender * group	Sphericity Assumed Greenhouse-Geisser	4 2.746	1.107 1.107	.360 .352
Error(Serum Na^+)	Sphericity Assumed Greenhouse-Geisser	72 49.432		
Serum K^+	Sphericity Assumed Greenhouse-Geisser	4 3.056	5.584 5.584	.001 .002
Serum K^+ * gender	Sphericity Assumed Greenhouse-Geisser	4 3.056	1.627 1.627	.177 .193
Serum K^+ * group	Sphericity Assumed Greenhouse-Geisser	4 3.056	15.284 15.284	.000 .000
K^+ * gender * group	Sphericity Assumed Greenhouse-Geisser	4 3.056	3.624 3.624	.010 .018
Error(Serum K^+)	Sphericity Assumed Greenhouse-Geisser	72 55.000		

that HbA1c and K^+ were higher, but HDL-cholesterol, total protein and albumin were lower in male addicts with non-insulin-dependent diabetes mellitus (NIDDM) versus non-addicted males with NIDDM. Total iron binding capacity (TIBC) and total protein were lower in addicts with NIDDM compared to non–addicted women with NIDDM (Asadi Karam *et al*., 2004). Our study was the first report that shows effects of opium on some serum factors in NIDDM male and female addicts. Although there are many reports on effects of opium derivatives such as morphine (Molina *et al*., 1994; Ipp *et al*., 1980; Ipp *et al*., 1978; Bossone and Hannon, 1991; Klaff *et al*., 1987; Kamei *et al*., 1998; Hashiguchi *et al*., 1995; Terner *et al*., 2003) noscapine (Landen *et al*., 2004; Ye *et al*., 1998; Mahmoudian and Mojaverian, 2001) and papaverine (Zacherl *et al*., 2003, 2004; Beltowski *et al*., 1998; Kraupp and Marz, 1995).

The effect of morphine on blood glucose has been demonstrated on animals (Molina *et al*., 1994; Ipp *et al*., 1980; Bossone and Hannon, 1991; Terner *et al*., 2003). Several mechanisms for its effects have been suggested, such as a rise in plasma adrenalin, noradrenalin, adrenocorticotrophic hormone (ACTH), cortisol (Bossone and Hannon, 1991) and glucagon (Molina *et al*., 1994) that have known as counter regulatory hormones, which increase blood glucose. Our findings on serum glucose in rats treated with opium are consistent with our previous study (Asadi Karam *et al*., 2004). As reported the effects of morphine remain around 6 hour (Glare and Walsh, 1991) and our results in rats indicated that at the 30, 60, 120 and 240 minutes it caused an elevation in serum glucose but at 360 minutes after opium injection the serum glucose was decreased. On the other hand counter regulatory hormones effect in different times, depends on kind of regulatory hormone, hence it is expected that alteration of glucose is time dependent. Our results about K^+ and Na^+ alteration is paralleled with the study that reported an increase in K^+ following morphine injection in pigs (Bossone and Hannon, 1991). It has also been reported that morphine's antinociceptive potency is gender dependent (Terner *et al*., 2003). Thus, it could be concluded that other effects of morphine are sex dependent. There is some evidence which show that gonadal hormone affect the response of the morphine (Steward and Rodaros, 1999), from this point of view our result about opium could be explained.

Papaverine, one of the other opium components increases renal flow, urine output (Zacherl *et al*., 2003, 2004), reduced Na^+-K^+-ATPase activity (Beltowski *et al*., 1998), and inhibits facilitated transport of nucleobases and sodium dependent activity nucleobases transport (Kraupp and Marz, 1995). Because of these roles of papaverine it could be concluded that papaverine plays an important role in Na^+, K^+-alteration in serum, and some finding about Na^+ and K^+ could attribute to papaverine actions. On the other hand morphine increases ACTH and cortisol (Bossone and Hannon, 1991), some results following opium injection about Na^+ and K^+ is due to this morphine property. Finally, opium contains more than 20 alkaloids, and more than 70 components (Venturella, 1995, Buchbauuer *et al*., 1994). More studies in molecular levels on cell line or animal models treated with opium (and opium derivatives) are warranted to have a better understanding of its mechanisms.

REFERENCES

Asadi Karam GR, Reisi M, Kaseb AA, Khaksari M, Mohammadi A and Mahmoodi M (2004). Effects of opium addiction on some serum factors in addicts with non-insulin-dependent diabetes mellitus. *Addiction Biology*, **9**: 53-58.

Barham D and Trinder P (1972). An improved color reagent for the determination of blood glucose by the oxidase system. *Analyst*, **97**: 142-145.

Beltowski J, Gorny D and Marciniack A (1998). The mechanism of Na^+- $K^{(+)}$-ATPase inhibition by atrial natriuretic factor in rat renal medulla. *J. Physiol. Pharmacol.*, **49**(2): 271-283.

Bossone CA and Hannon JP (1991). Metabolic actions of morphine in conscious chronically instrumented pigs. *Am. J. Physiol.*, **260**: 1051-1057.

Buchbauuer H, Nikiforov A and Remberg B (1994). Headspace constituents of opium. *Planta. Med.,* **60**: 181-183.

Glare PA and Walsh TD (1991). Clinical Pharmacokinetics of Morphine. *Therapeutic Drug Monitoring*, **13**: 1-23.

Gutstien HB and Akil H (2006). Opioid analgesics. *In*: Brunton LL, Lazo JS and Parker KL. The Pharmacological Basis of Therapeutics, 11th ed . MacGrow Hill, pp 547-584.

Hanson GR (1995). Analgesic, antipyretic and anti-inflammatory drugs. *In*: Gennard AR (editor). The Science and Practice of Pharmacy, 19th edn. Remington, Mack Publishing Company, pp.1197-1198.

Hashiguchi Y, Molina PE and Abumrad NN (1995). Modulation of metabolic effects of morphine-6-glucoronide by morphine-3-glucoronide. *Brain Res. Bull.*, **38**: 325-329.

Ipp E, Dobbs R and Unger RH (1978). Morphine and endorphin influence the secretion of the endocrine pancreas. *Nature,* **276**: 190-191.

Ipp E, Schusziarra V, Harris V and Unger RH (1980). Morphine-induced hyperglycemia: role of insulin and glucagon. *Endocrinology*, **107**: 461-463.

Kamei J, Sodeyama M, Ohsawa M, Kimura M and Tanaka S (1998). Modulation by serum glucose levels on morphine-induced antinociceptive effect in C57BL/KSJ-db/db mice. *Life Sci.*, **62**: 1-6.

Klaff LJ and Taborsky GJ (1987). Role of pancreatic somatostatin determining glucagon response to arginine and morphine. *Am. J. Physiol*., **252**: 751-755.

Kraupp M and Marz R (1995). Membrane transport of nucleobases: Interaction with inhibitors. Gen Pharmacol., **26**(6): 1185-1190.

Landen JW, Hau V, Wang M, Davis T, Ciliax B and Wainer BH *et al* (2004). Noscapine crosses the blood-brain barrier and and inhibits glioblastoma growth. *Clin. Cancer Res*., **10**(15): 5187-5201.

Mahmoudian M and Mojaverian N (2001). Effect of noscapine, the antitussive opioid alkaloid on bradykinin-induced smooth muscle contraction in the isolated ileum of the guinea-pig. *Acta. Physiol. Hung.*, **88**(3-4): 231-237.

Molina PE, Hashinguchi Y, Ajmal M, Maza M and Abumrad NN (1994). Differential homodynamic, metabolic and hormonal effects of morphine and morphine-6-glucoronide. *Brain Res.*, **664**:126-132.

Opium-Wikipedia, the free encyclopedia. In: http://en.wikipedia.org/wiki/Opium (4/10/2006).

Stephano GB, Goumon Y, Casares F, Cadet P, Fricchione GL and Rialas C *et al* (2000). Endogenous morphine. *Trends Neurosci,* **23**: 436-442.

Steward J and Rodaros D (1999). The effects of gonadal hormones on the development and expression of the stimulant effects of morphine in male and female rats. *Behav. Brain Res.,* **102**: 89-98.

Terner JM, Lomas LM, Smith ES, Barrett AC and Picker MJ (2003). Pharmacogenetic analysis of sex differences in opioid antinociceptionin rats. *Pain*, **106**: 381-391.

Venturella VS (1995). Natural Product. *In*: Gennard AR (editor). The Science and Practice of Pharmacy, 19th edn. Remington, Mack Publishing Company, pp.400-402.

Ye K, Ke Y, Keshava N, Shanks J, Kapp JA and Tekmal RR *et al* (1998). Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells. *Proc. Natl. Acad. Sci.*, USA, **95**: 1601-1606.

Zacherl J, Bock S, Feussner H, Erhardt W, Siewert JR and Stangl M (2004). Periarterial application of papaverine during laparoscopic donor nephrectomy improves early graft function after kidney transplantation in pigs. *Surg. Endosc.*, **18**(3): 417-420.

Zacherl J, Thein E, Stangl M, Feussner H, Bock S and Mittlbock M *et al* (2003). The influence of periarterial papaverine application on intraoperative renal function and blood flow during laparoscopic donor nephrectomy in a pig model. *Surg. Endosc*., **17**(8): 1231-1236.