EFFECT OF INTRATHECAL MORPHINE ON BLOOD GLUCOSE, GLUCAGON AND TISSUE GLYCOGEN IN RAT, COMPARISON WITH THE EFFECT OF XANTHAN GUM ON BLOOD GLUCOSE

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SUMMARY: The effects of intrathecal (i. t.) morphine (25-100 µg) and of the intraperitoneal (i. p.) xanthan gum (30 and 60 mg/kg) on blood glucose level were studied in non- fasted rats. Morphine produced a time and dose-dependent hypoglycemia, whereas xanthan caused a moderately decreasing effect on blood glucose. Morphine and xanthan appear to be acting by different mechanisms, although the hypoglycemic effects of morphine appear to be due largely to an increased glucose uptake by skeletal muscle. Xanthan has a slow onset of hypoglycemic action which may allow homeostatic mechanisms to intervene. On the other hand, glucagon was increased significantly after i. t. morphine (50 µg) both at 30 and 60 min. The hyperglucagonaemic response after morphine may be due to a direct opioid effect on pancreas. The i. t. administration of morphine (50 µg) caused a time-dependent decrease in liver and muscle glycogen levels in morphine-treated rats compared with saline-controlled rats. The data obtained in the present work point to active utilization of liver and muscle glycogen (glycogenolysis) in morphinised animals concomitant with hypoglycemia and increased glucagon levels.

Key Words: Morphine, xanthan gum, glucose, glucagon, glycogen.

INTRODUCTION

The potencies of several opioids appear to be decreased by increases in glucose concentration, both in vivo and in vitro (1). However, opioid administration has been demonstrated to produce an increase in blood glucose in several mammalian species (2,3). A few exceptions to the hyperglycemic effect of morphine have been reported, including the occurrence of a secondary hypoglycemia after an initial hyperglycemic response in fasted animals (4,5). In contrast, a preliminary study in our laboratory indicated a hypoglycemic response to morphine in rats when it was administered by the intraperitoneal (i. p.) route (6). Also, the intrathecal (i. t.) administration of morphine and its congeners, but not opioid peptides or other agonists of opioid, delta, kappa and sigma receptors, cause a profound hypoglycemia in mice and rats (7-12). This effect of morphine is stereo specific, is elicited only by the i. t. and i. p. routes of administration, and is caused only by supra-analgesic doses.

The hypoglycemic effect of i. t. morphine in mice appeared to be antagonized by naloxone (8). Because the mechanism for this effect of naloxone is unknown and the hypoglycemic effect of i. t. morphine occurred at doses that also produced marked behavioral effects (8), the possibility was considered that the hypoglycemic effect of morphine might be mediated by the antagonism of spinal glycine receptors or due to some other non opioid mechanism (9).

There are data pointing to a direct effect of opiates on the pancreas. Thus, the ability of morphine to increase glucagon in unanesthetized mice and rats after acute i. t. and i. v. administration respectively has been well documented (11,13). Also, morphine has been found to increase glucagon levels when administered peripherally to both normal and alloxan diabetic dogs (14), and an encephalon analog has been found to modulate pancreatic function in vivo (15). The mor-

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phine antagonist naloxone has been used to block centrally induced hyperglycemia and hyperglucagonaemia in dogs, providing evidence for a role of endogenous opioids in glucose regulation (16).

Doses of i. t. morphine that were markedly hypoglycemic also produced a depletion of hepatic and muscle glycogen in mice (9,12,17). Also, recent studies from our laboratory have demonstrated a marked depletion in liver glycogen to the i. p. administration of morphine in unanesthetized non fasted rats (6).

To gain further insight regarding sugar/opiate interactions in rat, the purposes of the present study were to compare the effects of i. t. administration of morphine on blood glucose with those of hypoglycemic agents administered systemically, such as xanthan gum. In addition, the changes which might appear in glucagon level was studied following the i. t. administration of morphine. The effect of i. t. morphine on the level of glycogen in the liver and muscle was also studied to determine whether morphine-induced hypoglycemia might be due to an interference with glucose mobilization mechanisms.

MATERIALS AND METHODS

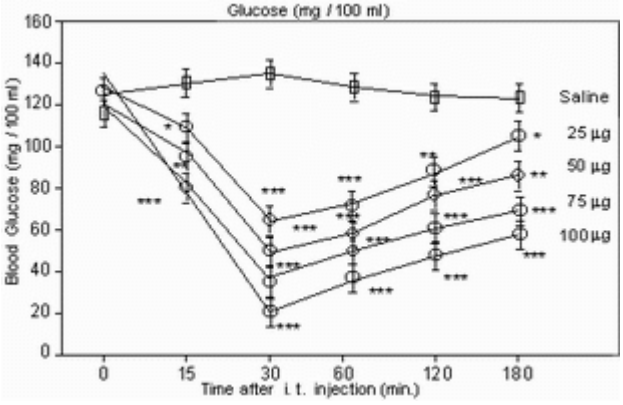
Animals: Male white albino rats (Rattus norvegicus) were obtained from the breeding unit of the National Research Center, Egypt. They weighed 160 -180 g at the time of use. The rats received food and water *ad libitum* and the experiments were performed between 9.00 and 11:00 a.m.

Chemicals: Morphine sulphate were obtained from Egypt Company for medical preparations, El-Mataria, Cairo, Egypt. Xanthan gum and most other chemicals were from the Sigma Chemical Co. (St. Louis, MO).

Injections: Percutaneous intrathecal (i. t.) morphine (25 - 100 mg) injections between the fifth and sixth lumbar vertebrae near the junction of the spinal cord and the cauda equina were made in unanesthetized animals in a volume of 5 μl, according to a modification (9) of the method of Hylden and Wilcox (18). The modification involved making the injection directly through the skin, rather than to precut the skin under general anesthesia. With this modification, a strong tail-flick reflex is elicited when the tip of the needle is in the proper position for making the injection. Rats not displaying the typical behavior of vigorous scratching at the site of injection within 3 min were not used, because the lack of this behavior indicated that the intended site of injection or the dose of morphine was not achieved. Drugs were dissolved in sterile 0.9% saline and control rats received the saline vehicle. Xanthan gum (30 or 60 mg/kg) was injected intraperitoneally (i. p.) in a volume of 10 ml/kg body weight.

Measurement of blood glucose, glucagon and tissue glycogen: Time courses (15 min-3 hr) of the effects of i. t. morphine on glucose and glucagon concentrations in serum and glycogen content in liver and muscle and the effects of i. p. xanthan gum on blood glucose concentration, were determined. Blood was withdrawn from the retro-orbital sinus with a 70-μl heparinized capillary tube before the injection of saline or drugs (morphine or xanthan) to establish a baseline glucose or glucagon levels and at the appropriate times after injection. Serum was isolated after the clotted whole blood was centrifuged at 3000 r.p.m. for 20 min. Serum specimens of each animal were divided into two parts in clean small tubes, the first part was kept at 4-8°C for analyses of glucose within 24 hr. The second part was quickly kept frozen at 20°C until needed for glucagon determinations. Glucose was measured using kits from Bio-Analytics Company (P.O. Box 388, Palm City, Fl. 34990). The levels of glucagon in blood serum was determined using the radio immunoassay (RIA) technique. RIA kits were provided by Diagnostic Products Corporation, 5700, west 96th Street (Los Angeles, CA 90045). At the appropriate time after injections, rats were decapitated. Immediately after sacrifice, tissues from liver and hind limb skeletal muscle also were excised and frozen. Glycogen in liver and hind limb skeletal muscle was assayed by colorimetric method of Seifter *et. al.* (19). Briefly, 1g liver or muscle was dissolved with heating in 3 ml of 30% KOH and then diluted with water. An aliquot was incubated with the anthrone reagent in sulfuric acid and the absorbance was read at 640 nm in a spectropho-

Figure 1: Time course and dose-response relationships for the effects of i.t. morphine on blood glucose in rats. Each point is the mean±SE of 6 animals *p<0.05; **p<0.01; ***p<0.001 vs. the saline group.



tometer. Glucose standards were carried through the same procedure to construct a standard curve.

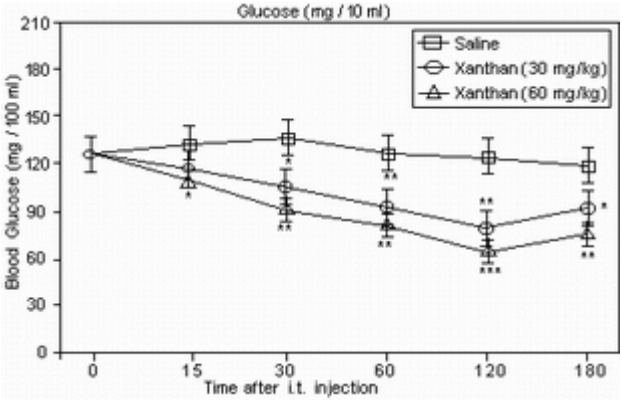
Statistics: Significant differences between groups were performed by analysis of variance. A p value less 0.05 was considered to be significant.

RESULTS

Effect of i. t. morphine on blood glucose

The effects of morphine, injected i. t. in doses ranging from 25 to 100 μg, on blood glucose levels were measured at various intervals up to 3 hours after injection. As shown in Figure 1, saline-treated control rats had an increase in blood glucose compared with the basal (pre-injection) level over the time period studies. Unlike mice (8), rats did not show significant hyperglycemic response to i. t. saline (+8.5% at peak). The i. t. administration of morphine caused a marked hypoglycemic response (Figure 1). Both the time of onset and the degree of hypoglycemia were dependent upon the dose of morphine. The maximal hypoglycemic observed at 30 min after the i. t. injection of 100 μg of morphine, the blood glucose level decreased by a mean of 81%. This dose of morphine caused a behavioral syndrome, consisting of scratching biting and vocalization, followed by seizures, as described in adult (20) and young (9) rats.

Figure 2: Time course and dose-response relationships for the effects of i.p. xanthan gum on blood glucose in rats. Each point is the mean ± SE of 6 animals *p<0.05; **p<0.01; ***p<0.001 vs. the control animals.



Effect of i. p. xanthan gum on blood glucose: The i. p. administration of xanthan in rats produced time and dose-dependent decreases in the level of blood glucose (Figure 2). Maximal hypoglycemia by a mean of 37% in treated rats than that of respective control animals, observed at 2 hours after xanthan (60 mg/kg) injection.

Figure 3: Serum glucagon level at various times in rats acutely injected i.t. with saline or morphine (50 μg). Each point is the mean ± SE of 6 animals *p<0.05; **p<0.01; ***p<0.001 vs. the pre-injected control animals.

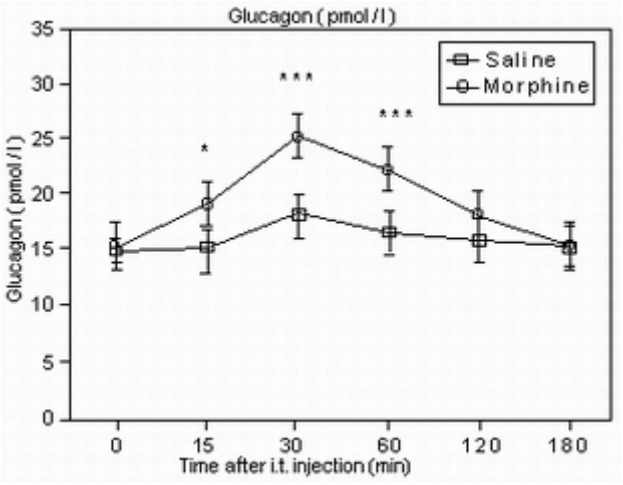
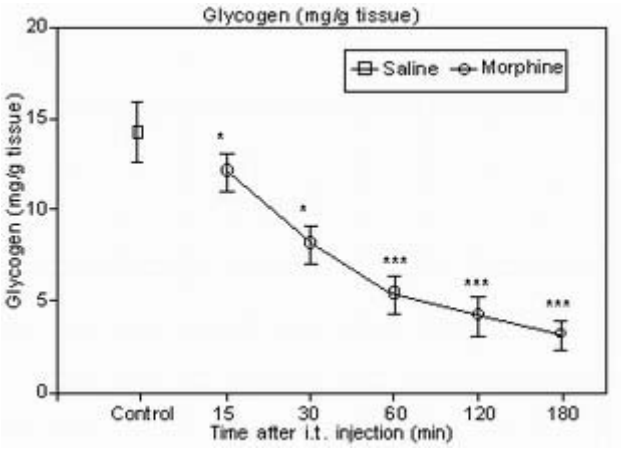
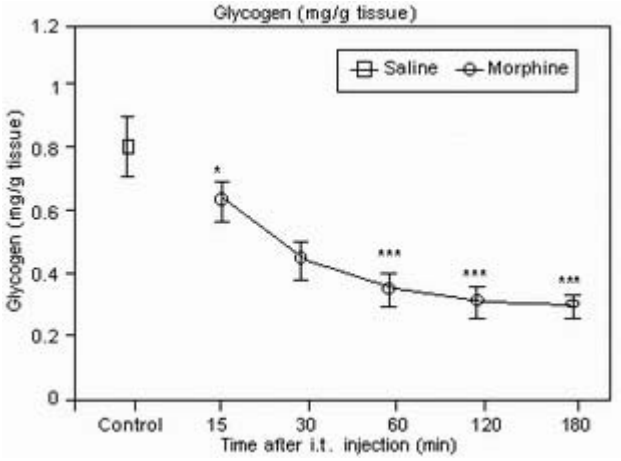


Figure 4: Liver glycogen content at various times in rats acutely injected i.t. with saline or morphine (50 μg). Each point is the mean ± SE of 6 animals *p<0.05; ***p<0.001 vs. the saline group.



Effect of i. t. morphine on glucagon: The effects of i. t. administration of morphine (50 μg) on glucagon levels were measured at various intervals up to 3 hours after injection (Figure 3). Glucagon was significantly increased at 30 minutes by a mean of 66% in treated

Figure 5: Muscle glycogen content at various times in rats acutely injected i.t. with saline or morphine (50 μg). Each point is the mean ± SE of 6 animals *p<0.05; ***p<0.001 vs. the saline group.



rats than that of respective pre-injected control animals. However, the glucagon level in morphinized animals was near the control values at 3 hours.

Effect of i. t. morphine on tissue levels of glycogen: Glycogen levels in muscle and particularly in liver from morphine-treated rats were substantially lower than those in the same tissues from saline-treated rats. Morphine (50 μg) produced over 76% depletion of hepatic glycogen by 3 hours after its i. t. administration (Figure 4). Also, the i. t. dose of morphine (50 μg) caused a mean decrease in muscle glycogen of 61% (Figure 5). Thus, although the nearly maximal hyperglycemia previously observed with a large subcutaneous (s. c.) dose of morphine (80 mg/kg) (8) was accompanied by a significant decrease (30%) in liver glycogen (9), a more profound glycogen depletion occurred after i. t. morphine (present study). These results indicate that the hypoglycemic effect of i. t. morphine is not simply due to liver glycogen depletion, because no hyperglycemia occurs before the hypoglycemic effect.

DISCUSSION

The effects of morphine were compared with this of one another hypoglycemic agent, xanthan gum. After administration of the hypoglycemic agent, the effect on blood glucose varies time wise with the agent, with xanthan acting much slower than morphine. Therefore, the level of blood glucose was determined after different pretreatment times, depending on the agent studied. As shown in Figures 1 and 2, the results of these studies in rats indicated differences among the effects of morphine and xanthan on the levels of blood glucose. The data clearly indicate that the i. t. administration of morphine produced a dose-dependent hypoglycemia. Similar observations were also reported in mice (8,9,12,17) and in rats (6,9). This hypoglycemic effect occurred without an initial hyperglycemic response, which distinguishes it from the secondary or compensatory hypoglycemia observed by Levine (4) in fasted rabbits after the s. c. administration of morphine or strychnine. The controversy in the literature is still evidenced where reports indicated that morphine had a hyperglycemic effect when administrated systemically or supraspinally (1,3).

Evidence from previous studies, however, indicates that i. t. morphine does not cause hypoglycemia by a change in urinary glucose excretion (8) or by causing the release of insulin (8,11). It does not increase serum levels of insulin immunoreactivity (11), and it has a robust hypoglycemic effect in mice with streptocotozin-induced diabetes (8). In contrast, insulin and xanthan did not have robust effects on blood glucose in ICR mice with insulin-deficiency diabetes (12). The last authors indicated that the hypoglycemic effect of i. t. morphine was accompanied by increased expiration of $^{14}CO_2$, increased muscle and kidney ^{3}H from $(^{3}H)_3$2-deoxyglucose and decreased liver and brain ^{14}C from (^{14}C) glucose. A large increase in ^{3}H without a comparable increase in ^{14}C indicated that morphine accelerated both the uptake and metabolism of glucose by certain tissues, especially skeletal muscle (12). Because exercise and anoxia are known to stimulate an insulin-independent activation of glucose transport in muscle (21,22), it is possible that seizures caused by high-dose. i. t. morphine (8,9) might contribute to its hypoglycemic effect. However, mice rendered tolerant to the hypoglycemic effect of morphine do not display tolerance to its convulsant effects (9) and hypoglycemia does not accompany seizures produced by the i.t. injection of other agents, such as strychnine, (-)-morphine-3-glucuronide, kainic acid and the (+)-enantiomer of morphine (9,10).

Our results revealed that xanthan gum decreased the blood glucose somewhat more than morphine. In support of our observations, White *et. al.* (12) reported that xanthan had less effect on the distribution of radio

labeled glucose and deoxyglucose could be due to its more moderate effect on blood glucose than that of morphine or insulin, its slow onset of action which may allow homeostatic mechanisms to intervene, or possible effects on glucose disposition to tissues. As an exopolysaccharide of bacterial origin (23), xanthan may exert a hypoglycemic action similar to that of lipopolysaccharide (LPS) from gram-negative bacteria, which stimulates glucose uptake by macrophage-rich tissues in vivo, including intestine, spleen, lung and liver (24,25). The lack of a significant xanthan induced increase in glucose uptake by whole liver (12) could be due to poor distribution of this viscous, high molecular weight polysaccharide from its i. p. injection site or lack of effect on hepatic parenchymal cells, as the effect of LPS on hepatic glucose uptake appears to involve primarily Kupffer cells, endothelial cells and infiltrated polymorphonuclear leukocytes, which constitute a relatively small portion of the total liver mass (26). LPS also has a slow onset of hypoglycemic action and is thought to act by stimulating the synthesis and release of interleukin-1 from macrophages which in turn mediates the hypoglycemia (27). A bi-directional cross-tolerance to the hypoglycemic effects of xanthan and LPS, indicating the possibility of a common mechanism of these two agents (12).

The i. t. morphine administration in the present work induced increases in glucagon level which are in agreement with those of Brase *et. al.* (11) and Johansen *et. al.* (13). The last authors demonstrated that the intravenous injection of morphine (10 mg/kg) induced glucagon release in rats which was accompanied by an increase in blood glucose. They also indicated that morphine administration induced an impressive insulin release. The inability of insulin to counteract the glucagon effect also makes it necessary to consider morphine-induced insulin resistance, or even a direct morphine effect on the liver (13). Also, in isolated dog pancreas IPP (28) has found morphine to stimulate both glucagon and insulin release. Reid and Yen (29) reported that i. v. administration of beta-endorphin induced release of glucagon and insulin together with hyperglycemia. In apparent contrast to this, Ipp (14) found no changes in blood glucose levels together with increases in glucagon and insulin levels after morphine administration in normal dogs. In our experiments both a direct opioid effect on pancreas and a reactive glucose-induced glucagon response may be inferred from the high glucagon levels observed after i.t. morphine.

The present study also demonstrated that the hypoglycemic effect of i. t. morphine was accompanied by depletion of liver and muscle glycogen. Similar observations were also reported in our laboratory in rats pretreated with i. p. morphine (6) and by Lux *et. al.* (9,17) and White *et. al.* (12) in mice pretreated with i. t. morphine. Opioid peptides were reported to stimulate glycogenolysis in isolated rat hepatocytes (30-32), but morphine did not share this effect in vitro (30). Considering also that negligible amounts of morphine would reach the liver after its i. t. administration, it is not likely that glycogen depletion in vivo represents a direct effect of morphine on liver glycogen stores or that such depletion represents the primary mechanism for the hypoglycemic effect of i. t. morphine. It is more likely that the depletion of glycogen occurred secondarily to an effect of morphine on the disposition of blood glucose. The decrease in blood glucose could activate both neurogenic and hormonal reflexes to stimulate glycogenolysis to maintain homeostasis of blood glucose (33,34). Lux *et. al.* (9) indicated that morphine caused a time and dose-dependent decrease in liver glycogen levels and was more potent in causing glycogenolysis than in causing hypoglycemia, because glycogenolysis would tend to counteract the hypoglycemic effect until a substantial decrease in glycogen occurred. This is supported by the previous observation that i. t. morphine was more potent in causing hypoglycemia in fasted mice than in fed mice (35). A dispositional mechanism for morphine-induced hypoglycemia is also supported by the fact that this effect also occurs in mice preloaded with large doses of glucose (8).

The glycogen-depleting effect of i. t. morphine also implies that the hypoglycemia is not due solely to a stimulation of the release of insulin, because insulin stimulates the synthesis of glycogen (36). This hypothesis is also supported by previous observation that i. t. morphine caused marked hypoglycemia in mice rendered diabetic by pretreatment with streptozocin (8), which destroys the insulin containing cells in the pancreas.

Our results are similar to those of White *et. al.* (12) who demonstrated that depletion in muscle glycogen would significantly occur in i. t. morphine treated mice. Thus, the stimulation of glycogenolysis by morphine

was not limited to hepatic glycogen, and the increase in glucose uptake by muscle was not accompanied by a parallel change in muscle glycogen.

It has been shown that the hypoglycemic effect of i. t. morphine in mice was blocked by acute spinal transection several segments above the lumbar site of injection (8,12), indicating that the hypoglycemic response requires an intact spinal pathway (6,9). Although the hypoglycemic effect of i. t. morphine required functioning spinal cord, it did not require presence of the adrenal glands (12).

In view of the above considerations, unlike insulin-(12) or xanthan-(present study) induced hypoglycemia, the hypoglycemic effect of i. t. morphine in the present work appears to be caused by a neurogenically mediated increase in glucose uptake by skeletal muscle and an increase in the metabolism of glucose to CO_2 *in vivo* (12). This neurogenic mediation does not involve the adrenals, but does require an intact spinal pathway.

It is concluded that the hypoglycemic effect of i. t. morphine is not due to interference with glucose mobilization from tissue glycogen as it rapidly depletes hepatic and muscle glycogen and significantly increases serum glucagon. These observations are consistent with the hypothesis that i. t. morphine-induced hypoglycemia is caused by activation of a neuronal pathway containing α_2-adrenoceptors and results in an insulin-independent acceleration of the rate of glucose uptake and metabolism by tissues especially skeletal muscle (12).

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