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EFFECT OF INTRATHECAL MORPHINE ON THE FATE OF GLUCOSE

COMPARISON WITH EFFECTS OF INSULIN AND XANTHAN GUM IN MICE

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Abstract—The hypoglycemic effect of morphine (40 μg) injected intrathecally (i.t.) was studied with regard to disposition of i.v. [^{14}C]glucose and [^{3}H]2-deoxyglucose and was compared with the effects of two other hypoglycemic agents, insulin (1 IU/kg, s.c.) and xanthan gum (50 mg/kg, i.p.). Mice given i.t. morphine or s.c. insulin exhaled a greater amount of $^{14}CO_2$ from i.v. [^{14}C]glucose than did control mice given i.t. saline, whereas there was less $^{14}CO_2$ expiration in xanthan-treated mice. In morphine-treated mice there was less ^{14}C in liver, brain and blood, and more ^{3}H in kidney and hindleg muscle than in control mice. Insulin-treated mice had more ^{14}C in muscle, less ^{14}C in liver, brain, kidney and blood, and less ^{3}H in liver and blood. In xanthan-treated mice, levels of both radiolabels were higher in liver, brain and kidney. Much lower glycogen content in muscle and depletion of liver glycogen occurred in morphine-treated mice, compared with control mice. Spinal transection completely inhibited the hypoglycemic effect of morphine, whereas adrenalectomy caused no inhibition. Morphine, insulin and xanthan appear to be acting by different mechanisms, although the hypoglycemic effects of both morphine and insulin appear to be due largely to an increased glucose uptake by muscle.

The intrathecal (i.t.)† administration of (−)-morphine and its congeners, but not opioid peptides or other agonists of opioid mu, delta, kappa and sigma receptors, causes a profound hypoglycemia in mice and rats [1–4]. This effect of morphine is stereospecific, is elicited only by the i.t. route of administration, and is caused only by supra-analgesic doses. It is antagonized at least partially by systemically administered naloxone, naltrexone and yohimbine, by i.t. naloxone methobromide, and by the induction of morphine tolerance in mice. The hypoglycemic effect of i.t. morphine also occurs in mice acutely loaded with glucose, in streptozotocin-diabetic ICR mice and in genetically diabetic mice [1].

Morphine-induced hypoglycemia is not accompanied by urinary glucose excretion [1] or by a significant change in serum insulin [4]. Morphine does not interfere with homeostatic glucose mobilization mechanisms, as it significantly increases plasma glucagon [4] and rapidly depletes hepatic glycogen [2]. 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. The purpose of the present study was to investigate the disposition of radiolabeled glucose and 2-deoxyglucose following the i.t. administration of morphine and to compare these effects of i.t. morphine with those of two hypoglycemic agents administered systemically, insulin and xanthan gum [5, 6]. Since i.t. morphine is increasingly being used as a spinal analgesic, it is important to understand any potential adverse effects of overdosage. Accordingly, the principal aim of this research was to ascertain the disposition of blood glucose after the i.t. administration of morphine.

MATERIALS AND METHODS

Animals. Male ICR mice and ICR adrenalectomized mice were obtained from Harlan Sprague–Dawley, Inc. (Frederick, MD). They weighed 20–35 g at the time of use. The mice received Agway RMH 3000 food pellets and water *ad lib.*, and the experiments were performed between 9:00 a.m. and 1:00 p.m.

Injections and spinalization. Intrathecal morphine injections were made in unanesthetized animals between the fifth and sixth lumbar vertebrae in a volume of 5 μL, according to a modification [2] of the method of Hylden and Wilcox [7]. Mice 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 (Baxter Healthcare Corp., Deerfield, IL) and control mice received the saline vehicle. Insulin and xanthan were injected s.c. and i.p., respectively, in a volume of 10 mL/kg body weight.

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† Abbreviations: i.t., intrathecal; and LPS, lipopolysaccharide.

Radioactive sugars were injected into a tail vein in a volume of 0.25 mL/25 g mouse. The 0.25 mL contained 25 μCi of ^{14}C and 150 μCi ^{3}H for the insulin and xanthan studies and half the radioactive sugars for the morphine studies. The timing to approximate the nadir of a hypoglycemic effect was 30 min, 10 min and 5 hr after drug treatment for morphine, insulin and xanthan, respectively. Spinalization of mice between vertebrae T6 and T8 was performed under halothane anesthesia according to the method described by Berge [8]. A small piece of Gelfoam (Upjohn Co., Kalamazoo, MI) was inserted into the cut before closing the wound with Autoclips (Clay–Adams Co., Parsippany, NJ). Sham-lesioned mice underwent cuts of the same amount of muscle and connective tissue. Spinalized and sham-lesioned mice were used 2 days following surgery. Spinalized mice that failed to show a normal tail-flick reflex to a thermal stimulus [9, 10] 2 days after surgery were not used.

Collection of CO_2. Immediately after injections of radiolabeled sugars, each mouse was placed into a 250-mL bottle, connected in series with three cylinders (125, 250 and 50 mL) fitted with fritted-glass aeration tubes. The first two cylinders contained 77 mL each and the third cylinder contained 22 mL of a 1:10 (v/v) mixture of TS-1 (0.6 N quaternary ammonium hydroxides in toluene) to trap expired CO_2 and Budget-Solve scintillation fluid (Research Products International, Mt. Prospect, IL). Air was drawn through this system with a water aspirator at a flow rate of about 150 mL/min. CO_2 was collected for 30 min in the morphine and xanthan experiments and for 15 min with insulin. A 20-mL aliquot from each cylinder was counted with a Beckman LS1701 liquid scintillation spectrometer after the addition of 200 μL of glacial acetic acid to quench chemoluminescence.

Measurement of blood glucose, tissue glycogen and radioactivity. Baseline glucose levels were determined in blood withdrawn from the retro-orbital sinus with a 70-μL heparinized capillary tube (Fisher Scientific, Pittsburgh, PA). At the appropriate time after injections, mice were decapitated and trunk blood was collected for measurement of glucose and radioactivity. Blood glucose was measured with Glucostix reagent strips and an Ames glucometer (model 5625; Ames Division, Miles Laboratory, Elkhart, IN). Upon decapitation, the head was frozen in a mixture of solid CO_2 and ethanol. Tissues from liver, kidney and hindlimb skeletal muscle also were excised and frozen. Glycogen in liver and hindlimb skeletal muscle was measured by the method of Hassid and Abraham [11]. The brain was removed and minced. A 100-mg portion of brain and 200-mg portions of other tissues were placed into paper cups (Packard Instrument Co., Downers Grove, IL) and covered with a small amount of cellulose powder (Whatman Biosystems Ltd., Maidstone, Kent, U.K.). Blood (200 mg) was weighed onto a cellulose pad in a paper cup. A few minutes before oxidation, 100 μL of Combustaid (Packard Instrument Co.) was added to each sample. Each tissue sample was burned for 1 min with a Packard Tri-Carb Sample Oxidizer (Packard Instrument Co.), and the resulting ^{3}H (as H_2O) and ^{14}C (as CO_2) were collected in scintillation vials containing Permafluor V scintillation fluid and CO_2-trapping fluid (Carbo-sorb and Monophase S). The recovered $^{14}CO_2$ and $^{3}H_2O$ were counted in a Beckman LS1701 liquid scintillation spectrometer.

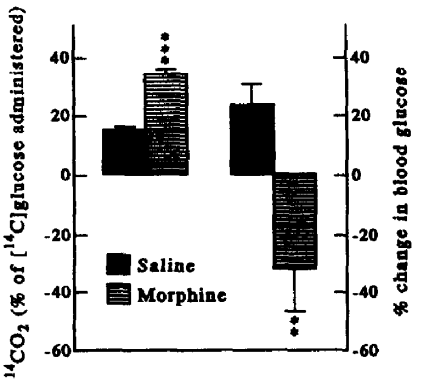

Fig. 1. Effect of i.t. morphine on expired $^{14}CO_2$ and blood glucose levels. [^{14}C]Glucose and [^{3}H]deoxyglucose were injected together i.v. 30 min after i.t. morphine (40 μg). Expired $^{14}CO_2$ was then collected for 30 min. Blood glucose was measured just prior to the i.t. injection of morphine and immediately following CO_2 collection. Values are means ± SEM (N = 7). Asterisks identify values significantly different from that of the control (**P < 0.005, ***P < 0.001). Baseline glucose in saline-treated mice was 137 ± 3.07 mg/dL; in morphine treated-mice, it was 141 ± 7.1 mg/dL.

Chemicals. Uniformly labeled [^{14}C]glucose (sp. act. 340 mCi/mmol) and [^{3}H]2-deoxy-D-glucose (sp. act. 30.2 Ci/mmol) were purchased from NEN (Boston, MA). Regular insulin (Iletin I) and morphine sulfate were products of Eli Lilly & Co. (Indianapolis, IN) and Mallinckrodt (St. Louis, MO), respectively. Anthrone for glycogen determinations was purchased from EM Science (Cherry Hill, NJ). Xanthan gum and most other chemicals were from the Sigma Chemical Co. (St. Louis, MO).

Statistics. The *t*-test for paired data was the preferred statistical method and was used for experiments in which a control mouse and a treated mouse were studied on the same day. With glycogen studies and studies of spinalized and adrenalectomized mice, the mice were studied in groups and, therefore, were compared with *t*-tests for grouped data.

RESULTS

Expiration of $^{14}CO_2$ *from* [^{14}C]*glucose.* Most mice had seizures during the hour after receiving i.t. morphine; blood glucose levels were lower and expired $^{14}CO_2$ was higher than in controls receiving i.t. saline (Fig. 1). Similar experiments were carried out with systemically administered insulin and xanthan. Although both insulin- and xanthan-treated mice showed significant reduction of blood glucose

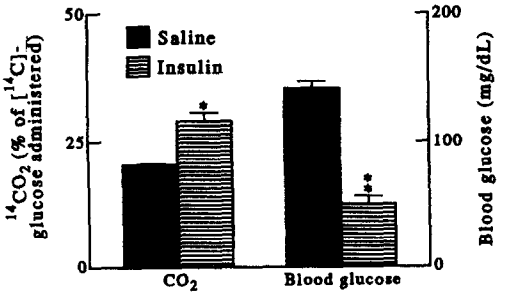

Fig. 2. Effect of insulin on expired $^{14}CO_2$ and blood glucose levels. [^{14}C]Glucose and [^{3}H]deoxyglucose were injected together i.v. 10 min after the administration of insulin (1 IU/kg, s.c.). Expired $^{14}CO_2$ was then collected for 15 min. Blood glucose was measured just after CO_2 collection. Values are means ± SEM (N = 5). Asterisks identify values significantly different from that of the control (*P < 0.01, **P < 0.005).

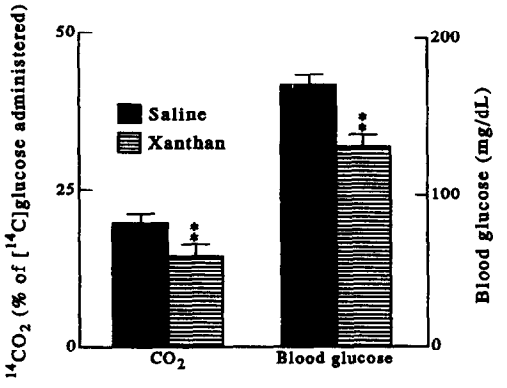

Fig. 3. Effect of xanthan on expired $^{14}CO_2$ and blood glucose levels. [^{14}C]Glucose and [^{3}H]deoxyglucose were injected together i.v. 5 hr after the administration of xanthan (50 mg/kg, i.p.). Expired $^{14}CO_2$ was then collected for 50 min. Blood glucose was measured just prior to the injection of xanthan and immediately following CO_2 collection. Values are means ± SEM (N = 5). Asterisks identify values significantly different from that of the control (**P < 0.005).

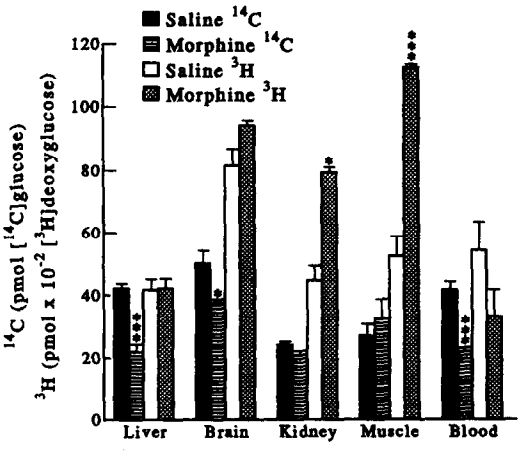

Fig. 4. Distribution of radioactivity from [^{3}H]deoxyglucose and [^{14}C]glucose after morphine. The mice and treatments were the same as those described in the legend to Fig. 1, except that N = 5. Asterisks identify values significantly different from that of the control (*P < 0.01, ***P < 0.001).

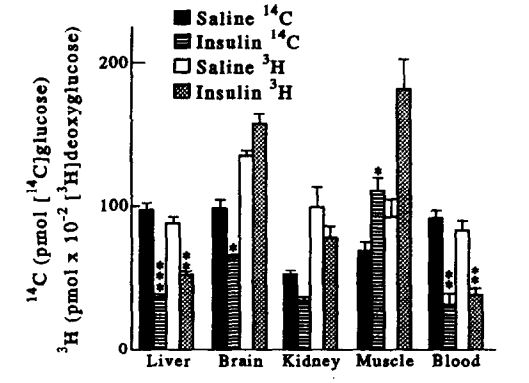

Fig. 5. Distribution of radioactivity from [^{3}H]deoxyglucose and [^{14}C]glucose after insulin. The mice and treatments were the same as those described in the legend to Fig. 2. Asterisks identify values significantly different from that of the control (*P < 0.01, **P < 0.005, ***P < 0.001).

(Figs. 2 and 3), their expired $^{14}CO_2$ was lower in xanthan-treated mice and higher in insulin-treated mice than that of respective control mice (Figs. 2 and 3).

Tissue levels of radioactivity. The tissue levels of ^{14}C from [^{14}C]glucose and ^{3}H from [^{3}H]deoxyglucose are shown in Figs. 4–6. Radioactivity from ^{14}C was significantly lower in liver, brain and blood of morphine-treated mice than in controls, whereas no significant differences from saline-treated mice were observed for ^{14}C in kidney and muscle. In contrast, ^{3}H was significantly higher in kidney and muscle but not significantly different in liver, brain and blood (Fig. 4). As observed in morphine-treated mice, insulin treatment caused significant decreases of ^{14}C in liver, brain and blood (Fig. 5). In addition, ^{14}C in muscle was increased significantly by insulin. Muscle ^{3}H tended to be higher and hepatic ^{3}H was significantly lower in insulin-treated mice than in saline-treated controls (Fig. 5). Xanthan caused higher ^{14}C in brain, but ^{14}C and ^{3}H distributions in other tissues were not affected significantly (Fig. 6).

Tissue levels of glycogen. Glycogen levels in muscle and particularly in liver from morphine-treated mice were substantially lower than those in the same tissues from saline-treated mice (Fig. 7).

Effect of spinal transection or adrenalectomy. Spinalectomized mice failed to show a hypoglycemic response to i.t. morphine. In contrast, sham-operated mice showed a hypoglycemic response to i.t. morphine similar to that exhibited by control mice (Fig. 8). Adrenalectomized mice also showed

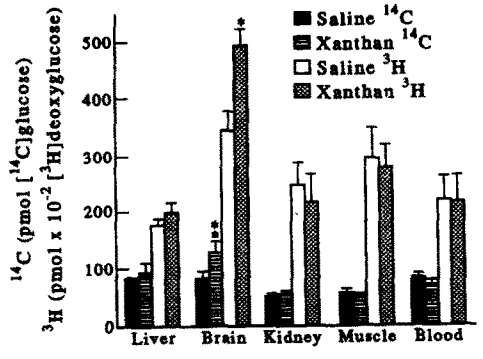

Fig. 6. Distribution of radioactivity from [^{3}H]deoxyglucose and [^{14}C]glucose after xanthan. The mice and treatments were the same as those described in Fig. 3. Values are means ± SEM (N = 6). Asterisks identify values significantly different from that of the control (*P < 0.01, **P < 0.005).

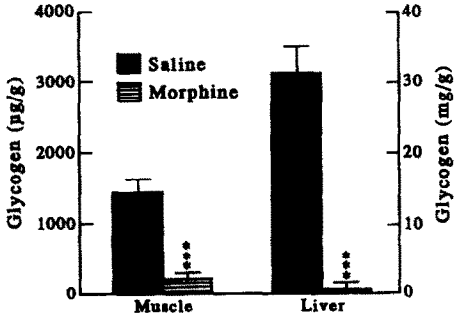

Fig. 7. Effect of morphine on muscle and liver glycogen levels. Tissues were obtained and frozen 1 hr after the administration of morphine (40 μg, i.t.) for later glycogen analysis. Values are means ± SEM (N = 5). Asterisks identify values significantly different from that of the control (***P < 0.001). Baseline blood glucose values were 141 ± 10 mg/dL for saline-treated mice and 136 ± 7.4 mg/dL for morphine-treated mice.

a typical hypoglycemic response to i.t. morphine, compared to normal control mice (Fig. 8).

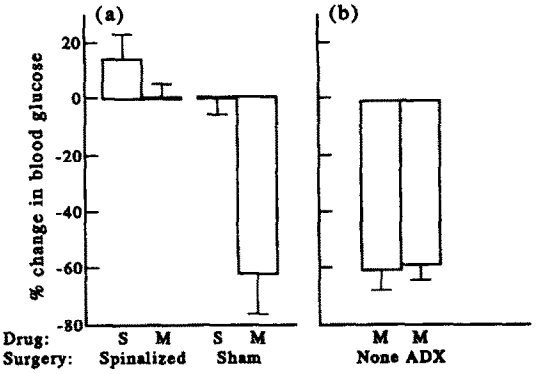

Fig. 8. Effect of morphine (40 μg, i.t.) on blood glucose (percent change from basal level) in (a) spinalized, sham spinalized and (b) adrenalectomized or normal control mice. "S" indicates saline-treated mice; "M", morphine-treated. Values are means ± SEM (N = 5 or 6). Blood glucose was measured just before and 1 hr after the i.t. morphine. Values are means ± SEM (N = 6). Baseline blood glucose levels were 135 ± 16, 149 ± 7.3, 162 ± 9.2, 163 ± 8.1, 136 ± 13 and 128 ± 7 mg/dL, respectively, for spinalized saline-treated, spinalized morphine-treated, sham saline-treated, sham morphine-treated, morphine-treated and adrenalectomized morphine-treated mice.

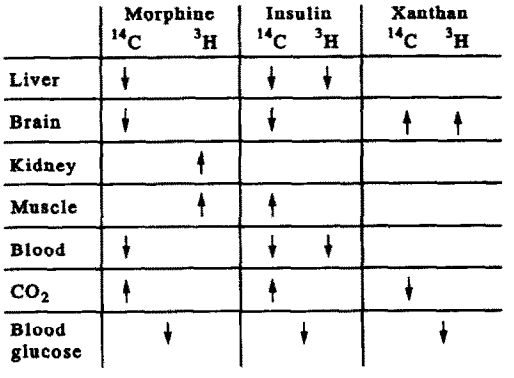

Fig. 9. Summary of the effects of morphine, insulin and xanthan on blood glucose levels, radioactivity from [^{3}H]-deoxyglucose and [^{14}C]glucose in various tissues, and on expired $^{14}CO_2$. The arrows indicate the directions of significant changes.

DISCUSSION

The effects of morphine were compared with those of two other hypoglycemic agents, insulin and xanthan. After administration of the hypoglycemic agent, the effect on blood glucose varies timewise with the agent, with insulin acting faster and xanthan much slower than morphine. Therefore, the disposition of labeled glucose and deoxyglucose was determined after different pretreatment times, depending on the agent studied. As summarized in Fig. 9, the results of these studies indicated differences among the effects of morphine, insulin and xanthan on the disposition of radioactivity from labeled glucose and 2-deoxyglucose. 2-Deoxyglucose is taken up by cells via the same membrane transport system that facilitates glucose uptake but, unlike glucose, 2-deoxyglucose is not further metabolized extensively after being phosphorylated by hexokinase [12]. The insulin-induced changes in mouse tissue levels of ^{3}H were similar to those reported by Hom and co-workers [13] in rats, except for the brain. The mouse brain level of ^{3}H was increased, whereas the rat brain level is decreased by insulin [13]. The reason for this discrepancy is not apparent, particularly since the mouse brain level of ^{14}C was decreased by insulin.

Except for the increase in kidney ^{3}H, morphine-

induced changes in the disposition of ^3H from [^3H]-2-deoxyglucose were similar to those caused by insulin, with the largest increases occurring in skeletal muscle, a well-established target tissue for insulin action [e.g. Ref. 13]. Evidence from previous studies, however, indicates that i.t. morphine does not cause hypoglycemia by causing the release of insulin [1, 4]. It does not increase serum levels of insulin immunoreactivity [4], and it has a robust hypoglycemic effect in mice with streptozotocin-induced diabetes [1]. In contrast, the doses of insulin and xanthan used in the present study did not have robust effects on blood glucose in ICR mice with insulin-deficiency diabetes.*

The hypoglycemic effect of i.t. morphine was accompanied by increased expiration of $^{14}CO_2$, increased muscle and kidney ^3H from [^3H]2-deoxyglucose and decreased liver and brain ^{14}C from [^{14}C]glucose. A large increase in ^3H without a comparable increase in ^{14}C indicated that morphine accelerated both the uptake and metabolism of glucose by certain tissues, especially skeletal muscle. Because exercise and anoxia are known to stimulate an insulin-independent activation of glucose transport in muscle [14, 15], it is possible that seizures caused by high-dose i.t. morphine [1, 2] might contribute to its hypoglycemic effect. However, mice rendered tolerant to the hypoglycemic effect of morphine do not display tolerance to its convulsant effects [2], 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 [2, 3].

The fact that xanthan had less effect on the distribution of radiolabeled 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 that were not examined in the present study. As an exopolysaccharide of bacterial origin [16], 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 [17, 18]. The lack of a significant xanthan-induced increase in glucose uptake by whole liver in the present study 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 [19]. 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 [20]. We recently observed a bidirectional cross-tolerance to the hypoglycemic effects of xanthan and LPS, indicating the possibility of a common mechanism of these two agents.

* Unpublished observations.

The present study confirmed that i.t. morphine causes a depletion of hepatic glycogen [2] and also demonstrated that muscle glycogen is decreased significantly. 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 was reported previously that acute spinal transection at T10/T11 inhibits the hypoglycemic effect of i.t. morphine [1]. Acute transection, however, may interfere with neuronal actions mediated caudal to the cut, as evidenced by the lack of a tail-flick reflex to a thermal stimulus. Allowing time after surgery for the recovery of the tail-flick reflex, however, did not result in the return of sensitivity to the hypoglycemic effect of i.t. morphine. Although the hypoglycemic effect of i.t. morphine required intactness of the spinal cord, it did not require the presence of the adrenal glands.

In conclusion, unlike insulin- or xanthan-induced hypoglycemia, the hypoglycemic effect of i.t. morphine 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*. This neurogenic mediation does not involve the adrenals, but does require an intact spinal pathway.

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