Diabetes Increases the Analgesia and Tolerance to Morphine in Acute Pain, but Not in Chronic Pain, While it Attenuates the Dependency in Rats

Joharchi KH, Jorjani M.

Neuroscience Research Center and Department of Pharmacology, Faculty of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, I.R. Iran

Changes in the concentration of either brain or blood glucose level appear to modulate antinociceptive and basal nociceptive processes. There are some contradictory data about the effect of diabetes on morphine antinociception. In this study the effects of alloxan-induced diabetes on morphine analgesia, tolerance and dependency were investigated, with a view to clarify the contradictions.

Materials and Methods: Experimental diabetes was induced by a single injection of alloxan (120 mg/kg S.C.) in rats. Administration of morphine sulfate (7 mg/kg i.p., 5 days) developed tolerance in animals. Acute and chronic pain in morphine treated diabetic and non-diabetic animals were evaluated using hot-plate and formalin tests respectively. In tolerant animals withdrawal signs (jumping, chewing, urine and feces) were recorded for ten minutes by the use of naloxone (2 mg/kg i.p.).

Results: Our results show that in the acute pain model, the antinociceptive effect of a morphine single dose was significantly enhanced (P < 0.001) in the diabetic group as compared to non-diabetic rats whereas, in diabetic tolerant rats in comparison with the non-diabetic tolerant ones morphine analgesia was extremely reduced (P < 0.001). In the chronic pain model after a single dose administration, the only antinociceptive potency of morphine was enhanced (P < 0.05) in the early phase, whereas after the induction of tolerance it was markedly reduced (P < 0.01) in the early phase; was slightly enhanced (P < 0.05) in the late phase in the diabetic rats compared to the non-diabetics. The withdrawal signs were significantly decreased (P < 0.001) in the diabetic compared to the non-diabetic animals.

Conclusion: It appears that the effects of hyperglycemia on pain thresholds differ in specific regions, using different tests and by duration of diabetes. Thus during the progress of diabetes, hyperglycemia might diminish the analgesic effect of morphine, blunt the development of dependency and alter the induction of tolerance.

Key Words: Diabetes, Morphine analgesia, Tolerance, Dependency, Rat

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Introduction

Earlier studies have demonstrated that the increase in blood glucose concentration whether induced or due to diabetes possibly affects neural mechanisms such as pain transmission and alters the potency of certain
narcotic analgesics such as morphine.\textsuperscript{1-4} This supports the hypothesis that glucose might interfere with morphine antinociception, tolerance and physical dependency. Drug tolerance and dependence represent experience-dependent changes in the central and peripheral nervous systems.\textsuperscript{5-7}

The increase in blood glucose concentration or hyperglycemia in diabetes profoundly alters hypothalamic-pituitary function, including the activity of endogenous opiate system.\textsuperscript{8} Changes in the concentration of either brain or blood glucose levels appear to modulate antinociceptive and basal nociceptive processes.\textsuperscript{9} Both clinical and animal studies show that morphine has a limited analgesic effect in the treatment of painful diabetic neuropathy.\textsuperscript{10-16} There are however some contradictory data on the effect of diabetes on nociceptive responses in laboratory animals.

Some investigators have reported a higher level of pain threshold in streptozotocin (STZ)-induced diabetic male rats via the hot plate device.\textsuperscript{17} Likewise, Levine et al. demonstrated prolonged tail-flick latencies to radiant heat in diabetic mice.\textsuperscript{18} and Akunne & Soliman have done the hot plate test and reported that diabetic animals have a significantly higher pain threshold than non-diabetic ones. It was also observed that glucose-induced hyperglycemic rats have a significantly higher pain threshold.\textsuperscript{19}

Conversely, others have reported that experimental diabetes mellitus attenuates the antinociceptive effect of morphine in rats and mice.\textsuperscript{20-25} They found that antinociception induced by morphine injected either systematically or intracerebroventricularly (i.c.v.) was attenuated in STZ-diabetic mice. Also Lee et al. showed that elevated blood glucose levels contribute to a decrease in pain thresholds of alloxan-diabetic rats,\textsuperscript{26} and a significant decrease in tail-flick latency in both diabetic and hyperglycemic control animals.\textsuperscript{27} Furthermore Morley et al.\textsuperscript{3} demonstrated a lowered pain threshold in humans rendered acutely hyperglycemic by glucose injection but, could not demonstrate a significant change in pain thresholds of diabetic patients. In many studies diabetic rodents have been shown to exhibit analgesic tolerance to morphine,\textsuperscript{12,23,25,28,29} and resistance to morphine dependence.\textsuperscript{5,6,30} Therefore, diabetic animals would be expected to be tolerant to the analgesic effects of exogenous opiates.

Thus this study was performed to resolve some aspects of the conflicting evidence relating to the effect of diabetes on morphine antinociception in acute and chronic pain models and to clarify the role of diabetes on opiate tolerance and dependency.

\textbf{Materials and Methods}

\textbf{Animals:} Male Sprague-Dawley rats weighing (180-250 g.) were used; animals were housed in animal room at 22±2°C with a 12h light/dark cycle and free access to water and food. Each animal was used only once.

All experiments were performed in accordance with the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983)\textsuperscript{31} and the Institutional Animal Welfare Law. All study protocols were approved by the internal deputy for animal research and the respective local government committee which is advised by an independent ethics committee in our Neuroscience Research Center.

Rats were divided into four main groups: diabetic, non-diabetic and their vehicle control groups. Each subgroup consisted of 6 rats.

\textbf{Drugs:} Alloxan, (Sigma, U.S.A.), 120 mg/kg/s.c., dissolved in distilled water.
Naloxone, (Sigma, U.S.A.), 2 mg/kg/i.p., dissolved in distilled water.
Morphine sulfate, (Temad, I.R. of Iran.), 7 mg/kg/i.p., dissolved in distilled water.
URIYAB-8 tape-test (Bakhtar Cheimi, I.R. of Iran).

\textbf{Induction of experimental diabetes:} Diabetes was induced by a single injection of alloxan (120 mg/kg s.c.) dissolved in 1 ml of
distilled water. Forty eight hours after administration, the urine glucose was measured colorimetrically using the URIYAB-8 tape-tests. Samples with urine glucose level less than 300 mg/100 were not included in this study. The urine glucose levels before the single dose of morphine were ≥ 300 mg/100mL and before the last dose of morphine were ≥ 1000 mg/100mL.

**Induction of tolerance:** Morphine sulfate (7 mg/kg i.p.) was administered for 5 consecutive days to induce tolerance in animals. Tolerance was defined as a significant decrease in analgesia between single dose and repeated doses of morphine injections.

**Measurement of nociceptive effect:** Hot-plate test was used to evaluate the acute pain. Animals were placed on a hot plate (55±5°C) according to the procedure described by Eddy and Leimback.32 The reaction time(s) measured was either hind paw licking or jumping off the plate. The cut-off point imposed was 60 seconds to avoid tissue damage.33 Each rat was tested twice in the first day, once before administration of morphine and next 30 minutes after the injection. The reaction time was subsequently assessed 30 minutes after morphine administration on the fifth day.

Chronic pain was assessed by the formalin test. First, morphine sulfate 7 mg/kg was injected i.p. for a single dose or for five consecutive days. Then on the first day (single dose) or on the fifth day (chronic dose), 45 minutes after the morphine injection, 50 µl of 2.5% formalin was subcutaneously injected into the plantar region of the right hind paw with a 27-gauge needle for noxious stimulation. The rats were placed in an open plexiglass chamber with a mirror positioned on the opposite side to allow unhindered observation of formalin-injected paw. Pain related behavior was quantified by counting the incidences of spontaneous flinching/shaking of injected paw. Pain rating was recorded according to the behavioral categories: 0,1,2 and 3 as described by Dubuisson and Dennis.34 Pain scores were recorded every 15 seconds after the injection of formalin for one hour. Every 5 minutes was considered as one time block (12 time blocks for one hour). The results of the formalin tests are presented as mean of pain scores during the first 15 minutes (the early phase), and 20-60 minutes (the late phase) after formalin injection.

**Assessment of Naloxone precipitated withdrawal signs:** The magnitude of physical dependency in the experimental groups was assessed by observing the characteristic signs of naloxone-precipitated withdrawal-like syndrome. On the fifth day of the experiment, 30 minutes after the injection of morphine, the withdrawal syndrome was precipitated by intraperitoneal injection of naloxone HCl (2 mg/kg). Immediately after the injection of naloxone the rats were placed in a plexiglass chamber and the following behaviors were observed continuously for ten minutes: chewing, jumping, urination, and feces. For the evaluation of each sign the number of chews, jumps, urination and excretion of feces were counted for each rat.35

**Data analysis:** Results were expressed as the Means ± S.E.M. Statistical analysis was performed by ANOVA analysis of variances with Tuckey’s post hock test. Data for two individual means was also analyzed by student’s t-test. P≤0.05 was considered to be significant.

**Results**

**Hot Plate Test:** 1) At baseline, even before any morphine injection, the hot plate latency time (HPL) is a little higher in the diabetic group but there is no significant difference between diabetic (11.17±0.87) and non-diabetic animals (8.00±0.52) in this state. The same result (11.67±0.71 vs. 6.83±0.60) is observed between respective control groups (Fig.1.). 2) Single dose administration of morphine sulfate (7 mg/kg, i.p.) induced significant analgesia in both diabetic (44.67±3.83) and non-diabetic (22.50±0.99) rats in comparison to their baselines [F(11, 60)=65.830, P<0.001]. It is interesting that
analgesia, or increase in HPL, is significantly higher in the diabetic compared to the non-diabetic group [F (11, 60)=65.830, P<0.001].

3) After the induction of tolerance by chronic administration of morphine sulfate (7 mg/kg, i.p. for 5 consecutive days), the antinociceptive effect of morphine in diabetic group (14.50±1.18) is highly reduced, and becomes almost equal to non-diabetic (14.17±0.83). Thus tolerance to morphine analgesia in the diabetic group (P<0.001) is more than non diabetic animals (P>0.01) in acute pain [F(11, 60)=65.830] (Fig. 1).

Formalin test: Fig. 2A confirms the above results in the early phase of formalin test. Single dose administration of morphine (7mg/kg, i.p.) significantly [F (7,40)=36.685, P<0.001] reduces the pain score in both diabetic and non-diabetic rats compared to their control groups (0.64±0.07 vs. 1.53±0.08 and 1.10±0.14 vs. 1.94±0.07 respectively). This reduction in diabetic group is greater [F(7, 40)=36.685, P<0.05] than in non-diabetics. Induction of tolerance by chronic administration of morphine sulfate (7 mg/kg, i.p. for 5 days) significantly increased the pain score in both diabetic [F(7, 40)=36.685, P<0.001] and non-diabetic [F(7, 40)=36.685, P<0.05] rats compared to their single dose administration (2.09±0.08 vs. 0.64±0.07 and 1.55±0.11 vs. 1.10±0.14 respectively). This increase is higher in the diabetic group compared to non-diabetics [F(7,40)=36.685, P<0.01].
Fig. 2. Effects of diabetes on formalin-induced pain related behavior in rats; Single Dose: Morphine sulfate 7 mg/kg, i.p. on first day. Chronic Dose: Morphine sulfate 7 mg/kg, i.p. 5 consecutive days; The Early Phase is the mean of pain scores during the first 15 minutes, The Late Phase is the mean of pain scores from 20 to 60 minutes, Data are presented as Means ± S.E.M; A) P (ANOVA)<0.0001, F(7, 40)=36.685; B) P (ANOVA)<0.0001, F(7, 40)=27.076, * P < 0.05, ** P < 0.01, *** P < 0.001; N = 6 in each group.
Fig. 3. Effects of experimental diabetes on naloxone-precipitated withdrawal signs in morphine tolerated rats; Morphine sulfate was administered 7 mg/kg, i.p. for 5 consecutive days. At the fifth day, 30 minutes after the injection of morphine, the withdrawal signs (chewing, jumping, urine and feces) were counted for ten minutes by the use of naloxone (2 mg/kg i.p.). Data are presented as Means ± S.E.M - Chewing: P(ANOVA)<0.0001, F(3, 20)=41.613, Jumping: P(ANOVA)<0.0001, F(3, 20)=22.231, Urine: P(ANOVA)<0.0001, F(3, 20)=24.733, Feces: P(ANOVA)<0.0001, F(3, 20)=12.588, *** P < 0.001 in comparison with non-diabetic group; N = 6 in each group.

there is still a significant [F(7,40)=36.685, P<0.05] analgesic effect of morphine in the non-diabetic group compared to their controls (1.55±0.11 vs. 2.01±0.09) but, there is no significant difference between diabetic tolerant rats and their control groups (2.09±0.08 vs. 2.20±0.05). Similar to the results of HPL, in the early-phase of formalin test which indicates acute pain, the degree of tolerance in diabetic animals [F(7, 40)=36.685, P<0.001] is greater than non-diabetic [F(7,40)=36.685, P<0.05] ones.

In the late phase of the formalin test (Figure 2B) the antinociceptive potency of morphine single dose is the same in both diabetic and non-diabetic groups (1.33±0.10 vs. 1.37±0.12). The reduction of pain score is also the same in both groups in comparison with their respective control ones [1.33±0.10 vs. 1.92±0.06 & 1.37±0.12 vs. 2.21±0.05, F(7,40)=27.076, P<0.001 in both groups]. In chronic dose, morphine analgesia is enhanced (P<0.05, student’s T-test) in diabetic tolerant rats compared to non-diabetic tolerant animals (2.10±0.04 vs. 2.35±0.11). According to this figure the tolerance to analgesic effect of morphine in chronic pain is almost the same in diabetic and non-diabetic animals [F(7, 40)=27.076, P < 0.001 in both groups).

Withdrawal syndrome test: After the injection of naloxone (2 mg/kg, i.p.) all precipitated withdrawal signs are significantly
(P<0.001) decreased in diabetic tolerated animals compared to non-diabetic tolerated ones (Fig.3.). The results are as follows:

Chewing: 2.50±0.22 vs. 17.17±2.55, F(3, 20)=41.613, P< 0.001.
Jumping: 0.17±0.17 vs. 2.83±0.60, F(3, 20)=22.231, P< 0.001.
Urine: 0.33±0.21 vs. 2.00±0.26, F(3, 20)=24.733, P< 0.001.
Feces: 0.33±0.21 vs. 3.67±0.92, F(3, 20)=12.588, P< 0.001.

These behaviors are not seen in vehicle control groups.

Discussion

The results of the present study demonstrate that after a single dose administration of morphine, the pain threshold is significantly enhanced in the diabetic state compared to non-diabetic animals. This enhancement is seen both in the acute pain model (Fig.1), and in the first phase of the formalin test (Fig. 2A). In other words, in diabetic group, after a single injection, morphine analgesia is enhanced in acute pain. This finding is also supported by the early phase of the formalin test. It has been reported by other investigators that in the diabetic state, endogenous opiates are released acutely along with ACTH, in response to cell hypoglycemia.36 Raz and co-workers20 proposed that in diabetes, generally, the pain threshold is adequately maintained, despite the antagonistic effect of glucose, partly due to a compensatory increased secretion of endogenous opioid peptides. Thus, from the present work and evidence in literature, it appears that in the diabetic state an endogenous opiate system may be activated first and potentiates morphine analgesia (Fig.1). Kamei and associates35 demonstrated that a δ-opioid receptor-mediated endogenous antinociceptive system is enhanced in diabetic mice. They have also shown that diabetic mice are selectively hypersensitive to supraspinal μ-opioid receptor-mediated antinociception, but they are normally responsive to activation of δ and κ-opioid receptors.21 Thus in accordance to acute pain mechanisms which are mainly mediated by spinal opioid receptors, it can be assumed that in the early stages of diabetes the spinal mechanisms are mainly involved in response to acute pain and the opioid system is over activated due to cell hypoglycemia. It has been reported that the elevated serum glucose levels may be responsible for the changes in sensitivity of opioid receptor subtypes only during the incipient stages of diabetes.35 It was also observed that glucose-induced hyperglycemic rats had a significantly higher pain threshold.19 Thus it seems that acute hyperglycemia can affect pain transmission in spinal centers rather than supraspinal.

However after chronic treatment and the induction of tolerance, the antinociceptive effect of morphine in acute pain model (Fig. 1) in diabetic group was more reduced than in non-diabetic tolerated animals. Thus the diabetic group shows more tolerance to morphine analgesia. This is also seen in the early phase of formalin test (Fig. 2A). The comparison of single dose and chronic dose indicates that in the diabetic group, tolerance is more than, non-diabetic animals (Fig 2A). Thus in acute pain with chronic morphine treatment the tolerance is higher in the diabetic group; it can therefore be proposed that in diabetes the increase of blood glucose and the duration of hyperglycemia cause some pathologic mechanisms which may make changes in second/third messengers and/or ion channels.13 It may be due to the changes that occur in the first phase and influence the release of some chemical mediators or neurotransmitters such as glutamate,36 nitric oxide39,40 prostaglandins,41 substance P,42 noradrenalin,43 etc. Also the number of opioid receptors may be reduced,2 or their sensitivity to response to morphine may be changed.21 Since a difference between central and peripheral mechanisms is observed, we can assume that in early diabetes the κ and δ opioid receptors which are hyper-responsive, are involved but in late diabetes the hyporesponsive μ-opioid receptors may be in-
involved. This difference may also be due to the changes in signaling pathways of opioid receptors which can reduce the active attachment of agonist to its receptor. Anyhow it appears that there are many different pathways involved in this process, and all of these changes can induce hyperalgesia in diabetic group in a manner that the degree of tolerance to morphine analgesia in acute pain is even higher than in the non-diabetic group.

But, in the late phase of formalin test (Fig. 2B), morphine analgesia, after a single dose, is almost the same in the diabetic and non-diabetic groups. And the comparison of single dose with chronic dose in this figure indicates that the level of tolerance in both groups in chronic pain model is almost the same. Thus differences in literature could be due to using different pain models (acute or chronic), differing durations of morphine usage (single or repeated) or the time of study (2 days, 5 days, or more). In the early stage that the glucose level is about ≥ 300 mg/100, the insulin deficiency is not remarkable, the endogenous opiates are released and the pathologic mechanisms are not yet established, the increase in morphine analgesia is seen in diabetic group. But in the later stages of diabetes, with the increase of blood glucose (≥ 1000 mg/100 in the last day), the decrease of insulin and the activation of pathologic mechanisms, the tolerance to morphine analgesia is established in a way that acute pain tolerance is more than in non-diabetics but chronic pain tolerance is the same. Furthermore the antinociception of morphine in the diabetic group is slightly more than in the non-diabetics (Fig. 2B, chronic dose). Thus in the chronic pain model, after the induction of tolerance, the antinociceptive effect of morphine in the diabetic group is reduced in the early phase (Fig. 2A) but is enhanced in the late phase of formalin test (Fig. 2B). Therefore it is observed that diabetes can progressively reduce the acute pain threshold but can slightly enhance the chronic pain threshold. Also it seems that as diabetes proceeds and the interaction between hyperglycemia and opioid system continues the pain is modulated in supraspinal centers. Forman suggested that diabetes results in a progressive degeneration of the neuroendocrine system over time and many of the complications produced by diabetes develop over an extended period rather than an immediate response to hyperglycemia. It has also been reported that the antinociceptive effects of opioid receptor agonists in diabetic mice are altered in a region-specific manner in the central nervous system. They postulated that in the late stages of diabetes, the elevated serum glucose levels do not influence the attenuation of μ-opioid receptor agonist-induced antinociception in diabetic mice. Therefore the differences in data obtained may be attributed to the time course for the development of diabetic complications. Thus as diabetes proceeds the central mechanisms and the μ-opioid receptors are more involved and with the increase of blood glucose the μ-opioid receptors are adapted in a manner that seems to be hyper-responsive in chronic pain which is less tolerated in the diabetic group. This is supported by extremely significant reduction of withdrawal signs or dependency in diabetic group (Fig. 3). This finding is consistent with other reports. It can be assumed that chronic morphine injections can antagonize the blood glucose effects in pain processes in a manner that tolerance and dependency will be reduced in the late stages of diabetes. Kamei & Ohsawa, have reported that the functional changes in central noradrenergic systems may be involved in the reduction of naloxone-precipitated jumping in morphine-dependent diabetic mice. They have also shown that intracellular calcium has some role in the modifications of naloxone-precipitated withdrawal jumping in morphine-dependent mice. Some investigators have reported that K<sub>ATP</sub> channels have some role and the others suggested that nitric oxide, glutamate or substance P are involved.
Nevertheless it seems that when the animals are in a diabetic state, in the early stages the hyperactivity of spinal responses is involved but, as the diabetes proceeds the alteration of opioid receptor function in the supraspinal region is less affected by hyperglycemia than in the spinal region, and here increase in central responses are seen. Therefore the effects of hyperglycemia on pain threshold are different in specific regions, with different tests and by duration of diabetes. Thus with the progression of diabetes, hyperglycemia lowers morphine’s analgesic potency and increases the pain sensation and somehow hinders the production of morphine tolerance such that with the increase of glucose concentration in diabetes the sensation of pain is enhanced, and the dependency is reduced.

These findings support the hypothesis that hyperglycemia might diminish the analgesic effect of morphine, and blunt the development of dependency to this drug. Diabetes may also alter the induction of tolerance, by exerting a direct antagonistic effect on the opiate receptor, affecting receptor numbers (up regulation), or an alteration in its conformation or expression. The antagonistic effect of hyperglycemia might be overcome by hyper secretion of endogenous peptides, such as β-endorphin. In addition some neurotransmitters including glutamate and nitric oxide could be involved in this process. Further studies are needed to clarify the exact mechanisms that increase antinociceptive effect of morphine as a consequence of the early diabetes and decrease the antinociceptive effect of morphine as a consequence of the late diabetes.

Considering the results of this study we can suggest that in acute pain treatment, the use of hypertonic glucose may help reduce the morphine dosage in normal patients and also, lessen the withdrawal signs in addicted patients.

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