Is the Reduced Efficacy of Morphine in Diabetic Rats Caused by Alterations of Opiate Receptors or of Morphine Pharmacokinetics?

C. COURTEIX, P. BOURGET, 1 F. CAUSSADE, 2 M. BARDIN, 3 F. COUDORE, J. FIALIP and A. ESCHALIER 3

Equipe NPPUA, Laboratoire de Pharmacologie, Faculté de Pharmacie, F-63001 Clermont-Ferrand Cedex, France

Accepted for publication December 12, 1997 This paper is available online at http://www.jpet.org

ABSTRACT

Because it generally is admitted that neuropathic pain is resistant to opioid analgesia, we investigated the effect of morphine on hyperalgesia in streptozocin-induced diabetes in rats. The antinociceptive effect of morphine (0.5–4 mg/kg i.v.) on mechanical (paw pressure test), thermal (tail immersion test) and chemical (formalin test) hyperalgesia was reduced. To clarify the mechanisms involved in the alteration of morphine analgesia, the binding characteristics of mu and delta receptor agonists and the pharmacokinetics of morphine and its glucuronide metabolites were determined. Kd and Bmax values for [3H][D-Ala2,(Me)Phe4,Gly(ol)5]enkephalin and [3H][D-Pen2,D-Pen5]enkephalin. Gly(ol)5]enkephalin and [3H][D-Pen2,D-Pen5]enkephalin to cerebral mu and delta opiate receptors were not altered by diabetes. Likewise, the plasma maximal concentration of morphine and metabolites, as well as the area under the curve, did not differ between diabetic and normal rats. Only the total clearance and the apparent volume of distribution of morphine were increased in diabetic rats, which suggests that the diabetes-induced glucosylation of proteins might increase the distribution of morphine in the aqueous compartment. These data indicate that the reduced analgesic effect of morphine caused by diabetes cannot be explained by a decrease in opiate-receptor affinity or density but rather by kinetic alteration of morphine (increase of total clearance and of volume of distribution in comparison with healthy animals).

Neuropathic pain is an important complication of diabetes and clinical studies have reported the difficulty of managing it even though antidepressants have been shown to be partially effective. The response of chronic neuropathic pain to opioid treatment is controversial and according to some authors, neuropathic pain is intrinsically nonresponsive to opioids (Arné and Meyerson, 1988a, b), and to others, underdosing opioid medication may be responsible for this poor activity (Portenoy et al., 1990). Experimental models of diabetes have been described as relevant models of chronic pain with alterations of pain sensitivity (Calcutt et al., 1996; Courteix et al., 1993; Kamei et al., 1991, 1992; Lee and McCarty, 1990), less ability to depend physically on morphine (Shook and Dewey, 1986) and poor response to opioids administered systemically (Courteix et al., 1994; Raz et al., 1988; Simon and Dewey, 1981; Simon et al., 1981) or supraspinally (Kamei et al., 1992; Suh et al., 1996).

The mechanisms by which diabetes alters morphine potency are not clear, and few explanations have been proposed. Alteration of opiate receptor affinity for [3H]naloxone has been described in brain membranes from diabetic (db/db) mice (Morley et al., 1981). Dysfunction of the supraspinal mediation of opiate analgesia (Kamei et al., 1992) and decrease of the release of serotonin from the bulbospinal pathways (Suh et al., 1996) known to be activated by morphine (Widger and Wilcox, 1987) have been reported in diabetic rats. However, other hypotheses, poorly or never investigated in the streptozocin-induced diabetic rat model, could account for the reduced efficacy of opiates. Reduced ability of opioid drugs to bind to mu and/or delta opiate receptors could occur, which may be revealed by a decrease in the binding characteristics of appropriate ligands to opiate receptors, or modifications of the pharmacokinetics of morphine could occur, such as reduced production of M6G which possesses a greater potency than morphine as a mu opioid agonist (Abbott and Palmour, 1988; Gong et al., 1991, 1992; Pasternak et al., 1991).

ABBREVIATIONS: AUC, area under the curve; Cmax, maximal concentration; CI, total clearance; i.v., intravenously; MPE, maximum possible effect; MRT, mean residence time; MS, morphine-3-glucuronide; M6G, morphine-6-glucuronide; S.E.M., standard error of the mean; STZ, streptozocin; T1/2, elimination half-life time; Tmax, delay to reach Cmax; Vd, apparent volume of distribution; DAMGO, [D-Ala2,(Me)Phe4,Gly(ol)5]enkephalin; DPDPE, [D-Pen2,D-Pen5]enkephalin.
1987) or a lesser amount of the active drug because of an increase of its elimination.

First, this study compared the antinociceptive potency of morphine to relieve pain caused by mechanical (pressure), thermal (warm and cold) and chemical (formalin injection) stimuli in STZ-induced diabetic rats and normal rats. The use of various stimuli led to the determination of a complete spectrum of the antinociceptive effect of morphine taking into account that differences in the effect of opiates, according to the nature of the stimulation, have been described (Hill, 1994; Millan, 1986). Moreover, the different reactions assessed (i.e., paw withdrawal, tail withdrawal, paw elevation and paw licking) provided an opportunity to study the action of morphine at different sites of integration of pain (i.e., spinal or supraspinal). Second, to investigate possible opioid receptor changes induced by diabetes, the binding characteristics of \(^{3}H\)DAMGO and \(^{3}H\)DPDPE to mu and delta opiate receptors, respectively, were determined in diabetic and normal animals. Third, because the metabolite M6G is a long-lasting and powerful analgesic in animals (Gong et al., 1991) and humans (Abbott and Pamlour, 1988; Portenoy et al., 1992; Sullivan et al., 1989) and binds to mu and delta opiate receptors (Hucks et al., 1992), and because M3G, another metabolite of morphine, may antagonize the antinociceptive effect of morphine (Ekblom et al., 1993), we compared the metabolism of morphine in diabetic and normal rats. Furthermore, the usual plasma pharmacokinetic parameters were determined.

**Methods**

**Animals and Induction of Diabetes**

Male Sprague-Dawley rats (Charles River France, Cléon, France) weighing 200 to 250 g were used. They were housed three per cage under standard laboratory conditions, and given food and water ad libitum. Because some subjects might result from this experiment, the IASP Committee for Research and Ethical Issues Guidelines (Zimmerman, 1983) were followed.

The rats were rendered diabetic with an intraperitoneal injection of STZ (75 mg/kg) (Zanosar, Upjohn, St. Quentin en Yvelines, France) dissolved in distilled water. Diabetes was confirmed 4 weeks later by measurement of tail vein blood glucose levels with Ames Dextrostix and a reflectance colorimeter (Ames Division, Miles Laboratories, France). Only rats with a final blood glucose level \( \leq 14 \) mM were included in the study.

Control (normal) rats received only distilled water.

**Behavioral Study**

**Test procedures.** Mechanical stimulus: the paw pressure test. The rats were submitted to the paw pressure test described previously by Randall and Selitto (1957). Nociceptive thresholds, expressed in grams, were measured with a Ugo Basile alganiseges (Apellex; tip diameter of probe, 1 mm; weight, 30 g) by applying an increasing pressure to the left hind paw until withdrawal (cut-off was 750 g). The AUCs of the score variations were calculated by the trapezoidal rule to compare the global effect of morphine between normal and diabetic rats. The use of score variations (threshold values obtained for each experimental time-predrug threshold value) was justified by the difference in predrug threshold values between the two groups.

**Thermal stimuli: the tail immersion test.** Before and every 30 min after drug injections, the tail of the rat was immersed in a warm (40°C, diabetic and normal rat) and a cold non-noxious (10°C, only diabetic rats, to assess thermal alldynia) water bath until tail withdrawal or signs of struggle were observed (cut-off time was 15 sec). Before the test, each animal was adapted to be handled. Percent MPE was calculated from the following formula: MPE = (postdrug reaction time – predrug reaction time) \times 100/15 – predrug score).

**Chemical stimulus: the formalin test.** The rats were placed individually in plexiglass cages (40 \( \times \) 40 \( \times \) 40 cm) during 15 min to be adapted to the environment. Three mirrors were placed behind the lateral walls to facilitate the observation. Five minutes after drug injection, a subdermal injection of 50 \( \mu l \) of a formalin solution (5% in saline) was given into the dorsal surface of the left forepaw, and the animals were returned immediately to the chamber. Each animal was observed for 45 min. Pain-related behavior was categorized with respect to its severity with a numerical scale as described by Dubuisson and Dennis (1977). Four positions of the injected paw were gauged and affected by a numerical score as follows: 0 = the injected paw is pressed normally on the floor; 1 = the injected paw is allowed to make contact with the floor but little weight is placed upon it; 2 = the paw is kept off the floor; 3 = the injected paw is vigorously licked or chewed. An average pain intensity score ranging from 0 to 3 was then calculated, according to the weight-scores technique of Dubuisson and Dennis (1977) by multiplying the amount of time (in seconds) spent in each category by the numerical value, summing these products and dividing by the total interval time (180 sec). An analgesic drug would tend to elicit lower pain scores.

**Treatment protocol and experimental design.** Tests took place 4 weeks after the induction of diabetes. Only rats in which the reduction in pain scores at week 4 of diabetes was more than 15% of the value obtained in normal rats (except for the formalin test) were included. The animals were submitted to the nociceptive test (paw pressure or tail immersion) before drug injection. Once two stable threshold values were obtained, morphine or saline (NaCl, 0.9%) was injected i.v. in the caudal tail vein. Rats were arranged randomly in cages, each rat receiving either morphine or saline in the same volume (0.1 ml/100 g b.wt.).

Different doses of morphine were administered (0.5, 1, 2 or 4 mg/kg i.v.) and the experiments were performed blind by the method of equal blocks with randomization of treatments \( (n = 7 \) for each treatment). Different animals were used for each test and treatment.

**Statistical analysis.** Statistical significance was assessed with a two-way analysis of variance followed, when the \( F \) value was significant, by a Dunnett’s test to analyze the time course of the effect. Significant differences between normal and diabetic groups were determined by a Student’s \( t \) test. The significance level was .05.

**Binding Study**

**Preparation of membranes.** Diabetic and normal rats were decapitated and whole brains \( (\mu \text{mu-opioid receptors}) \) or cerebral cortex \( (\delta \text{delta-opioid receptors}) \) were removed rapidly and placed on ice. The first preparation step is common to the two types of structure. The cerebral structures were homogenized in 10 volumes \( (w/v) \) of ice-cold 50 mM Tris-HCl buffer (pH 7.7) with a Ultra Turrax homogenizer. For the study of mu opioid receptors, the final pellet was washed immediately and resuspended in 30 volumes of fresh Tris-HCl buffer and incubated at 37°C for 40 min to dissociate any receptor-bound endogenous opioid peptides. The homogenates were centrifuged again as described above. For the study of \( \mu \) opioid receptors, the final pellet was washed immediately and resuspended in 30 volumes of fresh Tris-HCl buffer to yield a final protein concentration of 0.5 mg/ml. For the study of \( \delta \) delta opioid receptors, the preparation was frozen \( (–70^\circ\text{C}) \) until the day of use. After thawing, the pellet was resuspended in 50 volumes of fresh Tris-HCl buffer to yield a final protein concentration of 0.1 mg/ml.

Protein concentrations were determined according to Lowry et al. (1951), with bovine serum albumin as standard.

**Receptor binding assay.** For the \( \delta \) delta receptors, membrane suspension \( (0.5 \text{ mg protein/ml}) \) was incubated with 1 to 60 nM \( ^{3}H\)DPDPE \( (25 \text{ Ci/mmol, Amersham, Les Ulis, France}) \) in the ab-
sence and presence of 10 μM naloxone (Sigma, St. Quentin Fallavier, France) for determination of nonspecific binding.

For μν receptors, membrane suspension (0.1 mg protein/ml) was coincubated with 0.3 to 11 nM [3H]DAMGO (60 Ci/mmol, Amersham, Les Ulis, France). Binding in the presence of 1 μM naloxone (Sigma, St. Quentin Fallavier, France) was used to determine the nonspecific binding.

Incubations were performed in triplicate in Tris-HCl buffer at 25°C for 60 min. The reaction was terminated by filtration through Whatman GF/B filters that were washed twice with 5 ml of ice-cold Tris-HCl buffer (50 mM, pH 7.7). Radioactivity was measured by liquid scintillation spectrometry using a Betamatic counter (efficiency of counting = 60%).

Data analysis. Specific binding was defined as total binding minus nonspecific binding. Data of saturation experiments were analyzed with the programs EDBA (McPherson, 1983, 1985) and LIGAND (Muson and Rodbard, 1980).

Pharmacokinetic Study

Sample collection. Diabetic (n = 9) and normal (n = 9) rats received an i.v. injection of 4 mg/kg of morphine hydrochloride. Blood samples were collected at different times (5, 15, 30, 60, 90, 120 min after the injection) by intracardiac puncture under light anesthesia (halothane, 0.01% thymol). All samples were centrifuged (3000 × g at 4°C for 10 min) and stored at −40°C until the assay.

Morphine and its metabolites assay. Samples were assayed by high-performance liquid chromatography with an ion-pair formation that complied with the analytical recommendations of some authors (Derendorf and Kaltenbach, 1986; Zoer et al., 1986; Konishi et al., 1990). Extraction conditions and limits of quantification for morphine were improved markedly. Morphine, N-ethylnormorphine (in vitro controls) and glucuronides (M3G and M6G) were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). Methanol (99.8%) and acetonitrile (99.8%) were filtered before use (HV 0.45-μm filters). Methanol was childproofed and from 5.3 to 8.7 μg for M6G and 101.64 to 1355.2 ng/ml for M3G.

The standards were prepared in pooled human plasma (12 drug-free volunteers) spiked with morphine and its related compounds at concentrations ranging as follows: 1.345 to 29.93 ng/ml for morphine, 6.235 to 103.92 ng/ml for M6G and 101.64 to 1355.2 ng/ml for M3G. Each plasma sample was assayed in duplicate, and analytical in vivo and in vitro controls were determined.

Pharmacokinetic study. Data were analyzed by a noncompartmental method (Rowland, 1980). The programs used were PharmPAC BD 1.0 and Siphar-Win. Terminal half-life (T1/2, hours) was calculated by the equation: T1/2 = ln 2/β. The area under the concentration-time curve from time zero to time 2 h of the last sample (AUC0–2h, ng/h/ml) was calculated by the trapezoidal rule and was extrapolated to infinity. The mean residence time (M.R.T., h) was determined by the formula of Yamaoka et al. (1978). The clearance (Cl, ml/min/kg) of morphine was calculated by the following equation: Cl/β = Dose/AUC0–→. The apparent volume of distribution (Vα/ Cl) was determined as follows: Vα = Cl/β, T1/2, Cmax and C2h were experimental values.

Results and statistical analysis. Plasma concentrations are expressed in nanograms per milliliter. Results are expressed as mean ± S.E.M. Statistical significance was assessed with a two-way analysis of variance, followed by a Dunnett’s t test to analyze the time course of the effect when the F value was significant. Significant differences between normal and diabetic groups were determined by a Student’s t test. The significance level was .05.

Effect of Morphine on Nociceptive Tests

Mechanical stimulus. Before morphine injection, withdrawal thresholds of normal rats (from 123.3 ± 4.8 to 134.3 ± 6.6 g) were significantly higher (P < .01) than those of diabetic rats (from 80.0 ± 2.9 to 82.9 ± 1.8 g). Morphine (2 and 4 mg/kg) dose-dependently and significantly (F = 3.254, P = .0009 and F = 4.813, P < .0001, respectively) increased thresholds in normal rats (fig. 1A). The maximum increase at 40 min was +60 ± 21 g and +152 ± 32 g for 2 and 4 kg/mg, respectively. This antinociceptive effect lasted 50 and 100 min after the injection of 2 and 4 kg/mg, respectively.

In diabetic rats a significant (F = 3.553, P = .0005) antinociceptive effect could be observed only at 4 mg/kg (fig. 1B). The maximum increase was +41 ± 16 g at 10 min, and the effect lasted 60 min after the injection.

The determination of the AUC bounded by the time-course curves of the variations of withdrawal thresholds (fig. 1C) confirmed the dose-dependent antinociceptive effect in diabetic and normal rats, and showed a significant difference between scores obtained in normal and diabetic rats for 4 mg/kg.

Thermal stimuli. Tail immersion in a 46°C water bath. Initial tail withdrawal delays were from 7.1 ± 0.5 to 8.7 ± 0.4 sec and from 5.3 ± 0.5 to 6.1 ± 0.4 sec in normal and diabetic rats, respectively. The significant difference (P < .01) between these scores confirms the diabetes-induced hyperalgesia (fig. 2, A and B).

Morphine dose-dependently increased the scores in normal rats (fig. 2A). Its antinociceptive effect corresponded to a MPE of 63 ± 19% for 1 mg/kg (F = 4.22, P = .0123), 79 ± 12% (P = 6.99, P = .0011) for 2 mg/kg and 88 ± 4% (F = 45.5, P < .0001) for 4 mg/kg at 30, 30 and 120 min after the injection, respectively.

In diabetic rats (fig. 2B), only the doses of 2 and 4 mg/kg induced a significant antinociceptive effect, maximal at 30 and 120 min, respectively (MPE = 52 ± 12% and 69 ± 4%, respectively).

Tail immersion in a 10°C water bath. Predrug values of...
the tail withdrawal threshold were from 5.6 ± 0.4 to 7.5 ± 1.2 sec in diabetic rats (fig. 3). Morphine at the two lowest doses (0.5 and 1 mg/kg) did not induce any significant effect. On the other hand, 2 mg/kg morphine induced a significant antialloodynic effect (F = 19.84, P < .0001) from 30 to 120 min. The MPE was 92 ± 8% 30 min after the injection.

Chemical stimulus. Formalin-induced hyperalgesia was significantly (P < .05) more marked in diabetic (fig. 4B) than in normal (fig. 4A) rats. The scores of the first 3 min were 1.57 ± 0.16 and 1.93 ± 0.07 in normal and diabetic animals, respectively. The duration of the first phase was longer in diabetics (0–18 min) than in normal (0–9 min) rats. In diabetic animals, hyperalgesia of the second phase was significantly more intensive (P < .05) from the 18th to the 27th min than in normal rats.

In normal rats, morphine (2 mg/kg) was ineffective on the acute phase but significantly decreased scores from the 27th to the 45th min post-formalin injection. Compared with values obtained in saline-treated rats, the decreases varied from 71 ± 19% to 86.1 ± 13.9%.

In diabetic rats, morphine significantly reduced the scores of the two phases. The reduction of the first phase was 24 ± 10% at 0 to 3 min. The maximal scores obtained were similar to those of normal rats injected with saline (1.47 ± 0.18 and 1.57 ± 0.16, for the first 3-min block, respectively). Morphine
In diabetic rats, and 4.63 ± 0.6 nM, respectively, and 151.3 ± 8.8 fmol/mg protein, respectively, in normal animals. The diabetic rats were not significantly different from those observed between diabetic and normal rats for metabolites are shown in table 1. No significant variation was significantly reduced the scores of the second phase of 51 ± 14%, 60 ± 15% and 74 ± 17%, 21 to 24, 33 to 36 and 42 to 45 min after the formalin injection, respectively. However, this effect is significantly lower (P < .05) than that observed in normal rats for the corresponding times.

**[^3H]DAMGO and[^3H]DPDPE Binding**

No significant difference was observed in the binding of[^3H]DAMGO to brain mu receptors between normal and diabetic rats. K_D values were 1.52 ± 0.26 nM and 1.54 ± 0.45 nM, respectively, and B_max values were 189.3 ± 9.4 fmol/mg protein and 163.3 ± 22.1 fmol/mg protein, respectively. The binding characteristics of[^3H]DPDPE to delta receptors in diabetic rats were not significantly different from those obtained in normal animals. The K_D and B_max values were 5.71 ± 0.62 nM and 151.3 ± 9.8 fmol/mg protein, respectively, in diabetic rats, and 4.63 ± 0.29 nM and 129.7 ± 10.7 fmol/mg protein, respectively, in normal rats.

**Pharmacokinetics of Morphine and Its Metabolites**

The pharmacokinetic parameters for morphine and its metabolites are shown in table 1. No significant variation was observed between diabetic and normal rats for C_{max}, T_{1/2z}, AUC and MRT values of either morphine or its metabolites. However, morphine Cl and V_d were increased significantly in diabetic rats, whereas no significant change was observed for its metabolites M3G and M6G between diabetic and normal animals.

**Discussion**

The present results in STZ-treated rats confirm that diabetes induces alterations of nociceptive thresholds and clearly indicate that morphine-induced analgesia is attenuated in diabetic animals, whatever the nature (mechanical, thermal, chemical) or the intensity (noxious or non-noxious) of the applied stimulation and the level of integration (spinal or supraspinal) of the response. This univocal result distinguishes this model from the model described by Bennett and Xie (1988). In this model of neuropathic pain in which a chronic constriction nerve injury produces allodynia and hyperalgesia, the efficacy of opioids is increased (Attal et al., 1992) with use of the tail-pinch (supraspinal response) and tail-flick (spinal response) tests in STZ diabetic mice. In the same way, an alteration of perialgesia, the efficacy of opioids is increased (Attal et al., 1994), 2-fold higher doses of morphine are necessary to increase withdrawal thresholds in diabetic rats in comparison with normal rats. This demonstrates that both spinal (paw withdrawal) and supraspinal (vocalization) responses are depressed in diabetic rats. This alteration of both spinal and supraspinal responses to pain was also by Kamei et al. (1992) with use of the tail-pinch (supraspinal response) and tail-flick (spinal response) tests in STZ diabetic mice. In the same way, an alteration of content and/or release of endogenous opioid peptides at both spinal and supraspinal levels as well as changes in their interaction with opiate receptors has been suggested in diabetic mice (Ramabadran et al., 1989). Taken together, literature data and the present results show that diabetes induces a generalized alteration that modifies the activity of morphine at the spinal and supraspinal level.

The reduced efficacy of morphine was confirmed by use of a thermal nociceptive stimulation (tail immersion in a 46°C water bath). The dose of 1 mg/kg was devoid of activity in diabetic rats, whereas it increased tail withdrawal latency in
normal rats. Furthermore, the MPE values obtained with 2 and 4 mg/kg morphine were significantly lower in diabetic (52 ± 12% and 69 ± 4%, respectively) than in normal rats (79 ± 12% and 88 ± 4%, respectively). The need to reach the dose of 2 mg/kg to alleviate thermal allodynia (cold stimulation) confirms the poor sensitivity to morphine of diabetic animals. For the dose of 2 mg/kg, the activity of morphine was more effective in countering allodynia (MPE = 92 ± 5%) than in reducing hyperalgesia caused by warm stimulation (MPE = 52 ± 12%). The impairment of morphine efficacy in thermal pain tests agrees with previous results of Simon and Dewey (1981) and Simon et al. (1981) which showed that acute or chronic hyperglycemia reduced the antinociceptive effect of morphine subcutaneously administered in the tail-flick test. Raz et al. (1988) by use of the hot plate test also showed a reduction of morphine analgesia in hyperglycemic rats. The intracerebroventricular injection of morphine resulted in an alteration of the response mediated by supraspinal mu opioid receptors measured by the tail-flick assay (Kamei et al., 1992; Suh et al., 1996). Finally, Ramabadran et al. (1989), with use of the same test but with a stimulus temperature of 50°C, demonstrated that the hyperalgesic response to naloxone was abolished in diabetic mice.

In the formalin test, the scores of the two phases were significantly greater in diabetic rats than in normal rats as described previously by Malmberg et al. (1993). Morphine significantly decreased responses in both the early and the late phase of the formalin test in diabetic rats. In normal rats, an antinociceptive effect was reached only on the late phase; the lack of effect of morphine on the early phase was inconsistent with literature data (Dubuisson and Dennis, 1977) even though morphine-induced score reduction of the first phase is usually lower than that of the second phase. Despite this discrepancy, the comparison between normal and diabetic rats clearly shows a significantly lower decrease in the second phase scores after morphine injection in diabetic rats than in normal animals.

To resolve whether prolonged hyperglycemia affects the affinity and the density of brain mu and delta receptors, we investigated the saturation curves of tritiated DAMGO and DPDPDE to membrane preparations from diabetic and normal rats. Whole brain and total cortex were used to give a general approach of the effect of STZ treatment on cerebral opioid receptors, assuming that if alterations occurred, they would be revealed on total brain or cortex. Thus, the reduction of the analgesic activity of morphine in diabetic rats cannot be explained by a variation of Kd and Bmax values for mu and delta opioid receptors, because no significant change is evident in comparison with Kd and Bmax values for both opioid receptor subtypes in normal rats. However, although the differences in mu and delta receptors were not statistically significant, the density of the mu binding sites was reduced by approximately 14% by STZ treatment. On the other hand, Bmax for DPDPDE on delta receptors seemed to be (but not significantly) increased by approximately 17%. These data suggest that the opioid receptor populations are imbalanced after STZ treatment. The use of another methodology (i.e., membrane pretreatment with Na+ and GDP and binding in presence of GDP), as recently described by Liu-Chen et al. (1995), could have revealed small changes in receptor binding. However that may be, our conditions are the same as used by Adams et al. (1987), who successfully have reported alterations (a 20–31% reduction) in mu binding sites in rat brain induced by b-funaltrexamine treatment. Finally, because our study carried out in whole-brain homogenate did not show changes of opioid receptor binding, it is also possible that uncoupling of G-protein with the mu opiate receptor and/or changes in mu opiate receptor-associated second messenger systems might result in the reduced effectiveness of morphine, as suggested by Mao et al. (1995) in an animal model of neuropathic pain.

Because the relationship between opioid concentration and analgesic efficacy is steep (Levine et al., 1983), the pharmacokinetic behavior of morphine, M3G and M6G, was considered in addition to opiate receptor exploration. In normal rats, the obtained morphine Cl, Vd and T1/2 values are similar to those reported by Hasselström et al. (1996). The amount of M6G formed after the administration of morphine is negligible (Hasselström et al., 1996) or absent (Coughtrie et al., 1989) in rats, compared with the amount of M6G found in humans (Coughtrie et al., 1989; Stain et al., 1995). In our study, morphine was converted to M3G as the major metabolite. However, the M3G and M6G kinetic parameters reported (Hasselström et al., 1989) were obtained after injection of each metabolite and consequently cannot be compared with ours.

In diabetic rats, some pharmacokinetic parameters of morphine such as Vd and Cl are modified. The increase of Vd might be caused by glycosylation of plasma proteins, reported previously in diabetic patients (Gwilt et al., 1991), which can alter the protein binding and increase the unbound fraction of morphine. Because morphine and glucuronides are hydrophilic compounds, high amounts may be attracted in aqueous compartments. Thus, it is possible that the amount of morphine in blood crossing the brain-blood barrier and present in the central nervous system is reduced. The existence of a relationship between the intensity of analgesia and the levels of morphine and its glucuronide metabolites in the cortical extracellular fluid (Barjavel et al., 1995) has been demonstrated by use of the microdialysis procedure. Consequently, a much greater fraction of morphine might reach the bio-phase in normal rats than in diabetic rats, which explains the difference of potency of the drug.

Total clearance of morphine also is increased. This probably is not related to variations of hepatic metabolism despite the previously described impaired metabolic activity of hepatocytes from STZ rats toward xenobiotics (Favreau and Schenken, 1988) but rather may be related to the well-known modifications of renal function described in humans (Gwilt et al., 1991), especially the increase of glomerular filtration occurring in early diabetes mellitus. The higher total clearance observed in diabetic rats than in normal rats clearly means that frequent doses will be required to maintain the same blood concentration in the two groups of animals as it is the rule in humans (Mather, 1987).

Thus, both the increase of morphine Cl, which results in a nonsignificant reduction of morphine AUC by approximately 37%, and the increase of morphine Vd, which results in a high diffusion of morphine in the aqueous compartment, can lead to a reduction of available morphine and to a decrease of its amount in the target organs, i.e., in the central nervous system. These two processes may explain the reduced effect of morphine, despite the unmodified T1/2. The examination of experimental values obtained from the paw pressure test and
plasma morphine levels tended to show that higher morphine levels would be necessary in diabetic rats to obtain the same pharmacological effect as observed in normal rats. The present study fails to show any change in morphine metabolism. For morphine, phase II reactions are the most important metabolic reactions, and glucuronidation is the major reaction in humans, but this process is species dependent (Xu et al., 1993). Taking into account (1) the increased β-glucuronidase activity observed in diabetes (Rao et al., 1989) and (2) the previously reported increase of glucuronidation of the hydroxylated metabolites of amitryptiline in STZ-induced diabetic rats (Coudéré et al., 1996), increases of M3G and M6G were expected, and the unchanged pharmacokinetics of morphine glucuronides observed in this study was surprising. Nevertheless, M3G/morphine and M6G/morphine AUC ratios obtained in diabetic rats (5.10 and 0.20, respectively) tends to be higher than those obtained in normal rats (2.93 and 0.16, respectively). It is possible that an increase of glucuronidation may occur but that it is compensated by the increase of morphine and/or glucuronide elimination. Because glucuronide pharmacokinetic parameters remain unchanged, this phenomenon does not influence morphine analgesic activity. However, in rats, despite contradictory reports, morphine glucuronidation, especially in the 6-position seems to be deficient (Oguri et al., 1990; Kuo et al., 1991) and eventual modifications may be not observed. Specific in vitro studies on morphine metabolism with hepatoctyes from STZ-diabetic rats and in vivo behavioral studies with direct administration of M6G and M3G would be necessary. Study of phase I metabolism was impossible because levels of oxidized metabolites, N-demethylated morphine and morphine-N-oxide, or of the O-methylated metabolite, codeine, were not assessed. However, the oxidation reaction minor pathways of morphine metabolism and the activity of the specific cytochrome P450 involved in the formation of codeine, i.e., cytochrome P2D6, were unchanged in human diabetes mellitus (Bechtel et al., 1988), contrary to those observed with other cytochrome P450 isoenzymes in STZ-diabetic rats (Favreau and Schenkman, 1988).

To conclude, the data reported herein confirm that diabetes-induced hyperglycemia alters pain sensitivity by inducing mechanical, thermal and chemical hyperalgesia and thermal allodynia. They show that this metabolic trouble reduced the antihyperalgesic and antiallodynic activity of morphine whatever the nature of stimulus applied and the level of integration of pain reaction. This reduced efficacy is caused by significant changes in the binding parameters for brain mu and delta opiate receptors. The meaningful explanation is in the pharmacokinetic alteration of morphine (increase in Vd and CL) which could lead to the reduction of morphine levels in central nervous system. However, further investigations are needed to determine the involvement of some endogenous systems that are involved in opiate analgesia. Then, the recent demonstration of the decrease in morphine antinociception caused by activation of N-methyl-D-aspartate receptors (Mao et al., 1995) and of the modulatory role of antiopioid peptides (neuropeptide FF or cholecystokinin) in acute and chronic pain states (Gouraridès et al., 1996; Stanfa et al., 1994), respectively would justify specific studies in diabetic rats.

References


Munson PJ and Rodbard D (1980) Ligand: A versatile computerized approach for computer programs for the IBM PC.


Send reprint requests to: C. Courteix, Laboratoire de Pharmacologie, Faculté de Pharmacie, Place H. Dunant BP 38, F-63001 Clermont-Ferrand, France.