J. Pharm. Pharmacol. 1989, 41: 496–498
Communicated October 24, 1988



# Hyperglycaemia as a factor affecting κ-opiate agonist-induced inhibition of the gastrointestinal transit in mice

K. RAMABADRAN, M. BANSINATH, H. TURNDORF, M. M. PUIG, *Department of Anesthesiology, School of Medicine, New York and University Medical Center, 550 First Avenue, New York, NY 10016, USA*

**Abstract**—The effects of highly selective κ-opiate agonists were assessed on the gastrointestinal motility in normoglycaemic and hyperglycaemic conditions in mice. Chronic hyperglycaemia was induced by streptozocin injection (200 mg $kg^{-1}$ i.p.), 7–8 days before the experiment. Acute hyperglycaemia was induced by glucose injection (5 g $kg^{-1}$ i.p.) at the time of opiate administration. The κ-opiate agonists, U-50488H and U-69593 (1, 3 and 10 mg $kg^{-1}$) were injected (i.p.) just before the charcoal meal. The animals were killed 45 min later and the distance travelled by the test meal was measured. In the normoglycaemic mice, both κ-agonists significantly ($P < 0.05$) inhibited the meal transit and this effect was significantly ($P < 0.05$) augmented in acute hyperglycaemic animals. However, in chronic hyperglycaemic animals U-50488H failed to inhibit the charcoal meal transit, while U-69593 produced anti-transit effect comparable to that observed in normoglycaemic mice. These results demonstrate that κ-opiate agonists produce anti-transit effects in mice and that these effects are enhanced during acute hyperglycaemia. The disparity of anti-transit effects of κ-opiate agonists in acute vs chronic hyperglycaemia supports the hypothesis that elevated glucose levels are not the primary mechanism for the altered response to opiates observed in the experimental models of diabetes.

Both pharmacodynamic and biochemical evidence for multiplicity of opiate receptors is now well established. Inhibition of gastrointestinal motility is one of the long-known classical effects of the opiate agonist morphine. Even though morphine is considered to be a prototype μ-receptor agonist, in-vivo studies implicate μ- as well as κ-receptors (Abbott et al 1986). Although the role of μ-receptors in the mechanism of opiate-induced inhibition of gastrointestinal transit is well accepted, the involvement of κ-receptors remain controversial. Using highly selective κ-agonists such as U-50488H, U-69593 and U-53445E [(+)-stereoisomer of U-50488H], we have recently reported that κ-agonists inhibit the charcoal meal transit in mice. Moreover, the selective κ-antagonist, MR 2266 was effective in reversing the anti-transit effects of the agonists (Ramabadran et al 1988), confirming the involvement of stereospecific κ-receptors in the opiate-induced inhibition of gut motility in mice.

Many exogenous and endogenous factors are known to affect the pharmacology of opiates. The significance of the changes in physiological parameters, such as temperature (Puig et al 1987 a; Bansinath et al 1988 a), blood pressure (Hutchinson et al 1986) and pH on the pharmacodynamics and/or pharmacokinetics of opiates has been recently investigated in our laboratory (Puig et al 1987 b; Bansinath et al 1988 a). Currently, elevated plasma glucose concentration (hyperglycaemia) as a factor affecting the response to opiates is under investigation. In hyperglycaemic states, an increase as well as a decrease in the analgesic potency of morphine has been reported (Davies et al 1956; Simon & Dewey 1981; Brase & Dewey 1988; Raz et al 1988). Oral, as well as intraperitoneal administration of glucose has been shown to attenuate morphine-dependence and withdrawal signs (Akunne & Soliman 1988). Some in-vivo (Shook & Dewey 1981; Simon et al 1981; Levine et al 1988; Lux et al 1988) and in-vitro (Shook et al 1986) experiments support the hypothesis that the decreased sensitivity to morphine in experimental models of diabetes is primarily due to the hyperglycaemia associated with diabetes.

Correspondence to: K. Ramabadran, Department of Anesthesiology, NYU Medical Center, 550 First Avenue, New York, NY 10016, USA.

However, some recent in-vivo (Ramabadran et al 1989) and in-vitro results (Brase et al 1987) are not in agreement with this hypothesis. Morphine-induced gastrointestinal inhibition (Bansinath et al 1988 b), thermic responses (Bansinath et al 1989 b) and mydriasis (Bansinath et al 1989 a) are not uniformly affected in acute and chronic hyperglycaemic conditions. Furthermore, hyperglycaemic conditions do not alter the analgesic potencies of certain opiate agonists like methadone, propoxyphene and meperidine (Simon et al 1981; Brase & Dewey 1988). Taken together, these results suggest that the hyperglycaemic conditions do not uniformly affect all opiates and/or all their pharmacodynamic responses.

The effects of hyperglycaemia on the pharmacodynamics of the opiate receptor sub-type selective agonists has not yet been reported. In the present study, the gut inhibitory effects of κ-selective agonists, U-50488H and U-69593 were assessed in normoglycaemic and acute and chronic hyperglycaemic mice. The results indicate that acute hyperglycaemia augments, while the chronic hyperglycaemia attenuates the anti-transit effects of κ-opiate agonists. The present study provides further evidence to the hypothesis that elevated glucose levels may not be the primary mechanism for the altered opiate response observed in the experimental models of diabetes (Bansinath et al 1988 b, 1989 a, b; Ramabadran et al 1989).

## Materials and methods

Male Swiss Webster mice, 25–30 g (Taconic Farms, PA) were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity and artificial light (06:30–19:00 h). The animals had free access to food and water and were used after a minimum of four days acclimatization to the housing conditions. Acute hyperglycaemia was induced by the injection of glucose (5·0 g $kg^{-1}$ i.p.). Chronic hyperglycaemia was induced by the injection of streptozocin (200 mg $kg^{-1}$ i.p.) (Sigma Chemical Co. St. Louis, MO). Streptozocin was dissolved in 0·01 M citrate buffer (pH 4·0–4·5) and administered 7–8 days before the experiment as reported earlier (Bansinath et al 1988 b, 1989 b). Control animals received citrate buffer alone. Blood samples were collected from the retro-orbital sinus to measure the blood glucose levels. Ames dextrostix and a reflectance colorimeter (Accu-chek II, Boehringer Mannheim Diagnostics, Indianapolis, IN) was used to measure glucose levels. In separate groups of mice, blood glucose levels were measured before, 15, 30 and 60 min after the glucose administration (i.p.) to monitor the temporal nature of blood glucose levels in acute hyperglycaemic mice.

U-50488H {*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide methanesulphonate hydrate} (Upjohn Co, Kalamazoo, MI) was dissolved in pyrogen free water just before use and was injected i.p. at the lower right quadrant region of the abdomen, in a volume of 10 mL $kg^{-1}$. The κ-agonist, U-69593 {[5*R*-(5, 7, 8β)]-(±)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide} was dissolved in pyrogen free water acidified with dilute acetic acid. The doses of κ-agonists used were 1, 3 and 10 mg $kg^{-1}$ which were based on the previous results from this laboratory (Ramabadran et al 1988). Animals in the control groups were injected

with the respective vehicle. The drug injections preceded the charcoal test-meal by 30–45 s. A minimum of 10 mice was used per group.

Gastrointestinal transit was measured by the charcoal meal test as described previously (Bansinath et al 1988 b; Ramabadran et al 1988). Before testing, the animals were fasted for 20–24 h with free access to water. They were given 0·25 mL of a suspension of charcoal meal (10% charcoal in 5% gum acacia) and 45 min later killed by cervical dislocation. The abdomen was opened and the intestine from the pyloric junction to the caecal end removed. The distance travelled by the charcoal meal as well as the total length of the intestine was measured. Gastrointestinal transit was expressed as the percentage of the distance travelled by the charcoal relative to the total length of the small intestine. The data are represented as group mean ± s.e.m. Statistical significance was assessed by two way analysis of variance (ANOVA) (dose × glycaemic condition) followed by the post-hoc comparison using Student-Newman-Keuls test where appropriate (CSS 1987). A value of $P < 0.05$ was considered to be statistically significant.

## Results

On the day of the experiment, streptozocin-treated animals had blood glucose levels of 450 ± 15 mg $dL^{-1}$, while citrate buffer-treated mice had levels of 136 ± 13 mg $dL^{-1}$. The blood glucose levels before and at 15, 30 and 60 min after i.p. glucose injections were, 140 ± 12, 230 ± 15 and 470 ± 5 mg $dL^{-1}$, respectively. The distance of charcoal meal transit, expressed as percent total gut length (mean ± s.e.m.) after vehicle and after different doses of U-50488H treatment in the normoglycaemic controls, and in acute and chronic hyperglycaemic groups, is provided in Table 1. In vehicle-treated normoglycaemic mice, the % of charcoal meal transit was 73 ± 2. Neither acute nor chronic hyperglycaemia itself affected the charcoal meal transit in vehicle-treated controls. The statistical analysis of the data indicated significant effect for the dose of the agonist ($F_{3,168} = 19{\cdot}118$, $P < 0{\cdot}001$) as well as for the glycaemic condition ($F_{2,168} = 30{\cdot}263$, $P < 0{\cdot}001$) and for the interaction ($F_{6,168} = 6{\cdot}731$, $P < 0{\cdot}001$). The corresponding results for the U-69593 treated mice are provided in Table 2. As in the case of mice treated with different doses of U-50488H, ANOVA indicated a significant effect for the dose of U-69593 ($F_{3,153} = 52{\cdot}328$, $P < 0{\cdot}001$), the glycaemic condition ($F_{2,153} = 19{\cdot}837$, $P < 0{\cdot}001$) and for the interaction ($F_{6,153} = 3{\cdot}667$, $P < 0{\cdot}01$). The post-hoc analyses (Newman-Keuls test) in normoglycaemic groups indicated that, when compared with respective vehicle-treated controls, both U-50488H and U-69593 produced significant inhibition of charcoal meal transit ($P < 0{\cdot}05$) (Tables 1, 2). Furthermore, the anti-transit effect of U-50488H was significantly augmented in acute hyperglycaemic animals, while it did not produce any inhibitory effect in chronic hyperglycaemic mice (Table 1). The anti-transit effect of U-69593 was significantly augmented in the acute hyperglycaemic mice, when compared with the normoglycaemic controls ($P < 0{\cdot}05$) (Table 2). However, the highest dose of U-69593 tested produced significant inhibition of gut-transit in chronic hyperglycaemic animals.

Table 1. Effect of U-50488H on charcoal meal transit in mice. Group I, II and III represents normoglycaemic controls, glucose- and streptozocin-treated mice, respectively. n ≥ 10 per dose.

| U-50488H mg $kg^{-1}$ (i.p.) | % transit (mean ± s.e.m.) I | II | III |
|---|---|---|---|
| 0 | 73 ± 2 | 75 ± 3 | 76 ± 6 |
| 1 | 67 ± 3 | 32 ± 5[a, b] | 64 ± 4 |
| 3 | 56 ± 2[a] | 48 ± 5[a, b] | 72 ± 5 |
| 10 | 52 ± 2[a] | 32 ± 5[a, b] | 73 ± 4 |

[a] $P < 0{\cdot}05$ when compared with the respective vehicle treated (dose = 0) animals.
[b] $P < 0{\cdot}05$ when compared with the respective normoglycaemic controls (group I).

Table 2. Effect of U-69593 on charcoal meal transit in mice. Group I, II and III represents normoglycaemic controls, glucose- and streptozocin-treated mice, respectively. n ≥ 10 per dose.

| U-69593 mg $kg^{-1}$ (i.p.) | % transit (mean ± s.e.m.) I | II | III |
|---|---|---|---|
| 0 | 73 ± 2 | 75 ± 3 | 76 ± 6 |
| 1 | 73 ± 2 | 58 ± 5[a, b] | 72 ± 3 |
| 3 | 55 ± 2[a] | 33 ± 5[a, b] | 67 ± 6 |
| 10 | 46 ± 2[a] | 22 ± 5[a, b] | 42 ± 4[b] |

[a] $P < 0{\cdot}05$ when compared with the respective vehicle treated (dose = 0) animals.
[b] $P < 0{\cdot}05$ when compared with the respective normoglycaemic controls (group I).

## Discussion

Recently, the pharmacology of κ-agonists is considered to be of great importance because they offer diversity both in chemical structure and antinociceptive effects. When compared with μ-agonists, the κ-agonists have limited side-effects on the gastrointestinal tract and respiration, and produce a milder form of physical dependence (Ramabadran 1985; Cowan & Gmerek 1986; Cowan et al 1988). The present study confirms our earlier report of the anti-propulsive effects of the κ-agonists in mice (Ramabadran et al 1988). In addition, the present results demonstrate that acute hyperglycaemia augments the anti-propulsive effects of the κ-agonists. This finding is qualitatively similar to the effects of acute hyperglycaemia on the anti-transit response to morphine observed in mice (Bansinath et al 1988 b).

Opiates are the analgesics of choice during perioperative periods. The post-operative period is often associated with acute hyperglycaemia (Diltoer & Camu 1988). Currently, κ-agonists are considered to be effective supplements to anaesthesia without liability of autonomic side effects of other available opioid analgesics (Althaus et al 1988). Albeit, κ-receptors are known to be involved in producing opiate side effects including respiratory depression and inhibition of gut motility (Hayes & Tyers 1983; Ramabadran et al 1988). Therefore, based on the present results indicating an augmented κ-opiate effect during acute hyperglycaemia, it will be of specific interest to assess the side effects during the post-operative period when κ-opiate agonists are used as analgesics.

The results on anti-propulsive effects of κ opiate agonists obtained in chronic hyperglycaemic mice deviate from that of acute hyperglycaemic groups and thus provide further evidence to the hypothesis that elevated glucose levels may not be the primary mechanism for the altered opiate response observed in the experimental models of diabetes (Bansinath et al 1988 b, 1989 a, b; Ramabadran et al 1989). In chronic hyperglycaemic mice, U-50488H did not produce any inhibition of gut-transit, suggesting that streptozocin-induced hyperglycaemia prevents the effects of κ-opiate agonists on gut motility. However, U-69593 produced an inhibition of gastrointestinal transit in streptozocin-treated mice. The differences in gastrointestinal effects of κ-agonists used can be explained on the basis of heterogeneity of κ-receptors (Iyengar et al 1986; Zukin et al

1988). Some studies indicate that cellular energy linked-processes, but not elevated glucose levels, are important in modulating the opiate analgesia (Singh et al 1983). The present study demonstrates that acute and chronic hyperglycaemia fail to uniformly modulate the κ-agonist effects on gastrointestinal tract and are in agreement with an earlier report on the antipropulsive effects of morphine (Bansinath et al 1988 b). The mechanism(s) involved in the augmented effects of κ-opiate agonists on gastrointestinal tract during acute hyperglycaemic states remains to be established. Further studies to assess κ-opiate-induced side effects during hyperglycaemic conditions will be of significance to develop the κ-agonists as ideal post-operative opiate analgesics with minimal side effects.

The authors thank Mr M. Lovitz for his help in preparing the manuscript and Dr P. F. VonVoigtlander of Upjohn company, Kalamazoo, MI, for his generous gifts of U-50488H and U-69593 used in this study.

## References

Abbot, F. V., Franklin, B. J., Libman, R. B. (1986) A dose-ratio comparison of mu and kappa agonists in formalin and thermal pain. Life Sci. 39: 2017–2024

Akunne, H. C., Soliman, K. F. A. (1988) Hyperglycemic suppression of morphine withdrawal signs in the rat. Psychopharmacology 96: 1–6

Althaus, J. S., DiFazio, A., Moscicki, J. C., VonVoigtlander, P. F. (1988) Enhancement of anesthetic effect of halothane by spiradoline, A selective κ agonist. Anesth. Analg. 67: 823–827

Bansinath, M., Turndorf, H., Puig, M. M. (1988 a) Influence of hypo-and hyperthermia on disposition of morphine. J. Clin. Pharmacol. 28: 860–864

Bansinath, M., Ramabadran, K., Turndorf, H., Puig, M. M. (1988b) Effects of acute and chronic hyperglycemia on morphine-induced inhibition of gastrointestinal transit in mice. Pharmacology 37: 281–285

Bansinath, M., Ramabadran, K., Turndorf, H., Puig, M. M. (1989a) Hyperglycemia does not modify the pupillary effects of μ and κ opiate agonists in mice. J. Ocular Pharmacol. 5: 33–43

Bansinath, M., Ramabadran, K., Ramanathan, S., Turndorf, H., Puig, M. M. (1989 b) Effects of acute and chronic hyperglycemia on morphine-induced hypothermia in mice. In: Lomax, P., Schonbaum, E. (eds) Thermoregulation: Research and Clinical Applications. Karger, Basel, pp 139–144

Brase, D. A., Dewey, W. L. (1988) Glucose and morphine-induced analgesia. In: Morley, J. E., Walsh, J., Sterman, B. (eds) Nutritional modulation of neural function. Academic press, New York, pp 263–268

Brase, D. A., Han, Y., Dewey, W. (1987) Effects of glucose and diabetes on binding of naloxone and dihydromorphine to opiate receptor in mouse brain. Diabetes 36: 1173–1177

Cowan, A., Gmerek, D. E. (1986) In-vivo studies on kappa opioid receptors. Trend. Pharmacol. Sci. 7: 69–70

Cowan, A., Zhu, X. Z., Mosberg, H. I., Omnaas, J. R., Porreca, F. (1988) Direct dependence studies in rats with agents selective for different types of opioid receptor. J. Pharmacol. Exp. Ther. 246: 950–955

CSS (1987) Complete statistical system with graphics and data management, Release 1A, Version B384, Statsoft Inc., Tulsa, OK.

Davis, W. M., Miya, T. S., Edwards, L. D. (1956) The influence of glucose and insulin pretreatment upon morphine analgesia in the rat. J. Am. Pharm. Ass. 45: 60–62

Diltoer, M., Camu, F. (1988) Glucose homeostasis and insulin secretion during isoflurane anesthesia in humans. Anesthesiology 68: 880–886

Hayes, A. G., Tyers, M. B. (1983) Determination of receptors that mediate opiate side effects in the mouse. Br. J. Pharmacol. 79: 731–736

Hutchinson, J. S., Ramabadran, K., Kong, M. L., Chan, J. Y. H. (1986) Investigating a possible relationship between pain sensitivity and hypertension. Ind. J. Pharmacol. 18: 68–72

Iyengar, S., Kim, H. S., Wood, P. L. (1986) Effects of kappa opiate agonists on neurochemical and neuroendocrine indices: evidence for kappa receptor subtypes. Life Sci. 39: 637–644

Levine, A. S., Grace, M., Billington, C. J., Gosnell, B. A., Krahn, D. D., Brown, D. M., Morely, J. E. (1988) Effect of morphine and nalmefene on energy balance in diabetic and non-diabetic rats. Pharmacol. Biochem. Behav. 29: 495–500

Lux, F., Brase, D. A., Dewey, W. L. (1988) Antagonism of antinociception in mice by glucose and fructose: comparison of subcutaneous and intrathecal morphine. Eur. J. Pharmacol. 146: 337–340

Puig, M. M., Warner, W., Tang, C. K., Laorden, M. L., Turndorf, H. (1987 a) Effect of temperature on the interaction of morphine with opioid receptors. Br. J. Anaesthesia. 59: 1459–1464

Puig, M. M., Bansinath, M., Vargas, M. L., Lovitz, M., Turndorf, H. (1987 b) Enhanced cardiovascular effects of morphine during hypothermia. Anesthesiology 67: A312

Ramabadran, K. (1985) An analysis of precipitated withdrawal in rats acutely dependent on morphine. Jap. J. Pharmacol. 37: 307–316

Ramabadran, K., Bansinath, M., Turndorf, H., Puig, M. M. (1988) Stereospecific inhibition of gastrointestinal transit by κ opioid agonists in mice. Eur. J. Pharmacol. 155: 329–331

Ramabadran, K., Bansinath, M., Turndorf, H., Puig, M. M. (1989) The hyperalgesic effect of naloxone is attenuated in streptozotocin-diabetic mice. Psychopharmacology 97: 169–174

Raz, I., Hasdai, D., Seltzer, Z., Melmed, R. N. (1988) Effect of hyperglycemia on pain perception and on efficacy of morphine analgesia in rats. Diabetes 37: 1253–1259

Shook, J. E., Dewey, W. L. (1981) Morphine dependence and diabetes. I. The development of morphine dependence in streptozotocin-diabetic rats and spontaneously diabetic c57BL/KsJ mice. J. Pharmacol. Exp. Ther. 237: 841–847

Shook, J. E., Kachur, J. F., Brase, D. A., Dewey, W. L. (1986) Morphine dependence and diabetes. II. Alterations of normorphine potency in the guinea pig ileum and mouse vas deferens and of ileal morphine dependence by changes in glucose concentration. Ibid. 237: 848–852

Simon, G. S., Dewey, W. L. (1981) Narcotics and diabetes. I. The effects of streptozotocin induced diabetes on the antinociceptive potency of morphine. Ibid. 218: 318–323

Simon, G. S., Brozelleca, J., Dewey, W. (1981) Narcotics and Diabetes. II. Streptozotocin-induced diabetes selectively alters the potency of certain narcotic analgesics. Mechanism of diabetes: Morphine interaction. Ibid. 218: 324–329

Singh, I. S., Chatterjee, T. K., Ghosh, J. J. (1983) Modification of morphine antinociceptive response by blood glucose status: Possible involvement of cellular energetics. Eur. J. Pharmacol. 90: 437–439

Zukin, R. S., Eghbali, M., Olive, D., Unterwald, E. M., Tempel, A. (1988) Characterization and visualization of rat and guinea pig brain κ opioid receptors: Evidence for $\kappa_1$ and $\kappa_2$ opioid receptors. Proc. Natl. Acad. Sci. USA. 85: 4061–4065