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Role of K_{ATP} channels in reduced antinociceptive effect of morphine in `streptozotocin-induced diabetic mice

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The nociceptive effect was measured using withdrawal latency in tail flick test in mice rendered diabetic by administering streptozotocin (200 mg/kg, ip). The antinociceptive effect of morphine (4 and 8 mg/kg, sc) and cromakalim, a K_{ATP} channel opener, (0.3,1 and 2µg, icv) was significantly reduced in diabetic mice. Moreover, co-administration of cromakalim(0.3µg) did not alter the reduced antinociceptive effect of morphine(4 mg/kg) in diabetic mice. Spleenectomy in diabetic mice restored the decrease in antinociceptive effect of morphine and cromakalim. Multiple dose treatment with insulin to maintain euglycaemia for 3 days in diabetic mice prevented the decrease in antinociceptive effect of morphine and cromakalim. However, hyperglycaemic tyrode's buffer did not alter the pD_2 value of morphine in isolated guinea pig ileum suggesting that hyperglycaemia does not interfere with µ receptor mediated responses *in vitro*. The results suggest that hyperglycaemia induced decrease in antinociceptive effect of morphine and cromakalim may be due to alteration in K_{ATP} channels. Some unknown factor from spleen in diabetic mice may be responsible for this alteration in K_{ATP} channels in diabetic mice.

Chemically induced diabetes mellitus in experimental animals leads to hyperalgesia[1-3] decrease in antinociceptive effect of morphine[4] and other more selective µ receptor agonists[5]. A downregulation of µ opioid receptors in diabetic animals may account for these observations but the results of radioligand binding studies do not support this contention[6].

Both, µ and δ opioid receptors are coupled to ATP-sensitive K^+ channels (K_{ATP} channels) in the central nervous system[7]. K_{ATP} channels are reported to be involved in mediating the antinociceptive effect of morphine[8-10]. Glucose induced glycolytic flux may close K_{ATP} channels by increasing ATP formation[11,12]. It may be probable that diabetes induced hyperglycaemia may alter the activity of K_{ATP} channels and this alteration in the activity of K_{ATP} channels may account for the decreased analgesic activity of morphine. Therefore, the present study has been designed to investigate the role of K_{ATP} channel in hyperglycaemia induced decrease in antinociceptive effect of morphine in diabetic mice.

Materials and Methods

Swiss albino mice (20-30 g) and Hartley guinea-pigs of either sex were used. They were housed in an animal house provided with 12 hr light/dark cycle and free access to water and food.

Isolated guinea-pig ileum preparation—Isolated guinea-pig ileum 20-30 mm in length was mounted in an organ bath containing Tyrode's buffer (NaCl 137m*M* ; KCl 2.7m*M* ; $CaCl_2$ 1.8m*M* ; $MgCl_2$ 0.1m*M*; $NaHCO_3$ 11.9m*M* ; NaH_2PO_4 0.4m*M* ; $C_6H_{12}O_6$ 5.55m*M*) maintained at 37°±1°C bubbled with air. pD_2 of morphine was calculated using KCl (75 m*M*) precontracted guinea-pig ileum. In separate set of experiments dose response curve of morphine was also plotted using a modified Tyrode's buffer containing high concentration of glucose (15 m*M*) to study the effect of acute hyperglycaemia on pD_2 of morphine. Time matched KCl precontracted ileum preparations were also put up to serve as control.

Experimental diabetes in mice—Diabetes was induced by a single ip injection of streptozotocin[5] (200mg/kg) dissolved in 0.5*N* citrate buffer (*p*H 4.5). Age matched mice were injected with citrate buffer to serve as control.

Blood samples were taken from retro-orbital sinus 2 and 4 weeks after the injection of streptozotocin. Blood glucose was measured colorimetrically at 600nm using o-toluidine method[13]. Mice with a fasting blood glucose level of more than 14m*M* were included in the study.

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Measurement of nociceptive threshold—Nociceptive threshold was measured by the tail flick test in mice[14]. The tail flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. Electrically heated nichrome wire was used as a source of radiant heat in the analgesiometer. The intensity of radiant heat was regulated in order to obtain pretreatment latency between 2 to 5 sec in both diabetic and non-diabetic animals. This adjustment of pretreatment latency to a fixed range has enabled us to compare antinociceptive effect of various drugs in non-diabetic and hyperalgesic diabetic mice. A cut off latency time was fixed at 10 sec. Tail flick latency was expressed as a percentage of the maximum possible effect (MPE):

$$\text{MPE}(\%)=\frac{(\text{Post treatment latency}-\text{Pretreatment latency}}{(\text{Cut off time}-\text{Pretreatment latency})}\times 100$$

Pretreatment latency refers to the control latency before the drug administration. Post treatment latency refers to the latency after drug administration.

Surgical spleenectomy—Swiss albino mice were lightly anaesthetised using pentobarbitone (25 mg/kg) and ether anaesthesia. A horizontal cut was given on the abdomen and spleen was surgically removed. Animals were allowed to recover for 48 hr before subjecting them to any drug treatment or experimental procedure. In sham operated animals, abdomen was cut open and sutured back without removing the spleen.

Experimental design—Twenty two groups of mice were employed in the present study.

Vehicle treated groups: Group I-IV (n=6 each) consisted of age matched nondiabetic and diabetic (administered streptozotocin 4 week earlier) mice which were administered normal saline 10 ml kg^{-1} sc and 10 µl of 5% DMSO icv respectively.

Morphine treated groups: Group V-VIII (n=6 each) consisted of age matched nondiabetic and diabetic mice which were administered 4 and 8mg/kg, sc morphine respectively.

Cromakalim treated groups: Group IX-XIV (n=6 each) consisted of age matched nondiabetic and diabetic mice which were administered 0.3, 1 and 2µg, icv cromakalim respectively before subjecting them to the tail flick test. Cromakalim was injected icv in conscious mice in a volume of 10 µl with Hamilton syringe as described by Haley and McCormick[15].

Morphine and cromakalim treated group: Group XV-XVI (n=6 each) comprised of non-diabetic and diabetic mice injected with a combination of 0.3µg, icv cromakalim and 4mg/kg, sc morphine respectively. Separate vehicle treated groups that received a combination of saline, sc and DMSO, icv were also included. However,% MPE of these groups was comparable to vehicle treated groups therefore data has not been presented separately.

Insulin treated groups: Group XVII-XVIII (n=6 each) consisted of diabetic mice whose hyperglycaemia was regulated by administering Zn insulin suspension (1U/kg every 8 hr) for 3 days before injecting them with 4mg/kg, sc morphine and 1µg icv cromakalim respectively.

Groups subjected to surgical spleenectomy: Group XIX-XXII (n=6 each) comprised of non-diabetic and diabetic mice subjected to surgical spleenectomy and were administered 4mg/kg, sc morphine and 1µg cromakalim icv respectively 48 hr after the surgery. Tail flick latency of all the above groups was measured immediately, 5, 15, 30, 45, 60, 90 and 120 min after the administration of the drug and the comparison between various drug treated groups was carried out at the time of peak MPE (%). The observer recording tail flick latency was not aware of treatment schedules.

Drugs

Streptozotocin and cromakalim were purchased from Sigma Chemical Co., St. Louis, MO, USA. Morphine sulphate was obtained from Jackson Laboratories, India and was dissolved in normal saline immediately before use. Streptozotocin was dissolved in 0.1*N* citrate buffer and cromakalim was dissolved in 5% DMSO. Insulin zinc suspension (lente) was purchased from Knoll Pharmaceutical Ltd., India.

Data analysis

All the results are expressed as mean ± SE. One way ANOVA followed by Dunnett's test was employed to calculate the statistical significance in case of multiple comparisons with control group only. One way ANOVA followed by studentised range test was used to calculate the statistical significance in case of multiple comparison between the various groups. Individual comparisons between diabetic and nondiabetic groups were made using independent Student's t test. Level of significance (α) was fixed at $P< 0.05$.

Results

Mice treated with streptozotocin had fasting blood glucose level more than 14 m*M* whereas citrate buffer (0.1 *N*) treated controls had a fasting blood glucose levels of 5.0± 0.4 m*M*. Morphine administered (sc) produced an increase in tail flick latency time expressed as% MPE in both non-diabetic and diabetic mice at both the doses employed in the study. Moreover, streptozotocin induced diabetes significantly decreased morphine induced increase in % MPE as compared to control non-diabetic mice (Fig. 1).

Cromakalim administered icv to non-diabetic mice produced significant increase in% MPE at all the three dose levels as compared to vehicle treated control. On the other hand, cromakalim administered to streptozotocin diabetic mice also increased tail flick latency but results were only statistically significant high dose (2 µg) of cromakalim (Fig. 2). Cromakalim induced increase in% MPE was significantly less in case of diabetic mice as compared to non-diabetic controls at all dose levels (Fig. 2).

Moreover, co-administration of cromakalim (0.3 µg, icv) with morphine (4 mg/kg, sc) slightly increased % MPE in non-diabetic mice but no such increase in % MPE was noted in diabetic mice (Fig. 3). Three days insulin treatment to restore the elevated blood glucose levels in diabetic animals to normal produced marked recovery of antinociceptive effect of morphine (Fig. 4) and cromakalim (Fig. 5) in diabetic mice. However, the regain in antinociceptive effect of morphine and cromakalim with insulin treatment was partial and increase in % MPE in insulin treated animals was significantly less as compared to non-diabetic mice. The antinociceptive effect of morphine and cromakalim was significantly increased in diabetic mice subjected to spleenectomy and increase in% MPE in spleenectomised diabetic animals was comparable to that of non-diabetic group. It is to be noted that the morphine and cromakalim induced increase in% MPE in spleenectomised diabetic mice was higher as compared to insulin treated diabetic mice but the difference was not statistically significant (Figs 4, 5).

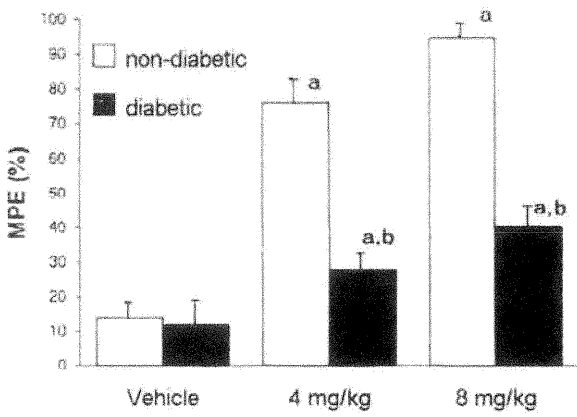

Fig. 1—Effect of morphine on percent MPE (mean ±SE, n=6) in non-diabetic and diabetic mice. [a=P<0.05 as compared to vehicle treated control, (ANOVA followed by Dunnet test, F=73.82 non-diabetic, F=17.51 diabetic); b=P<0.05 as compared to its non-diabetic counterpart. (Independent Student's t-test)]

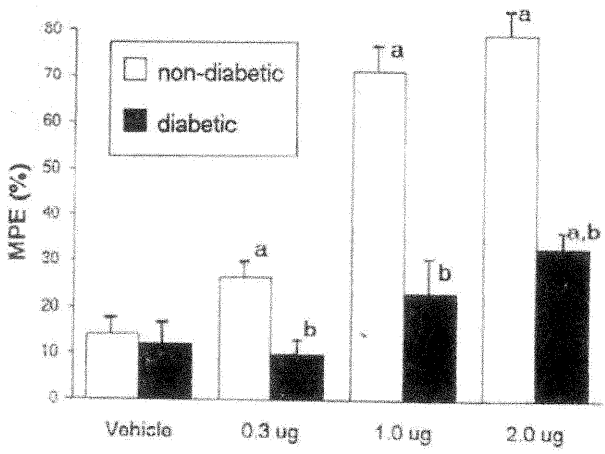

Fig. 2—Effect of cromakalim on percent MPE (mean ±SE, n=6) in non-diabetic and diabetic mice. [a=P<0.05 as compared to vehicle treated control, (ANOVA followed by Dunnet test, F=42.43 non-diabetic, F=4.6 diabetic); b=P<0.05 as compared to its non-diabetic counterpart. (Independent Student's t-test)]

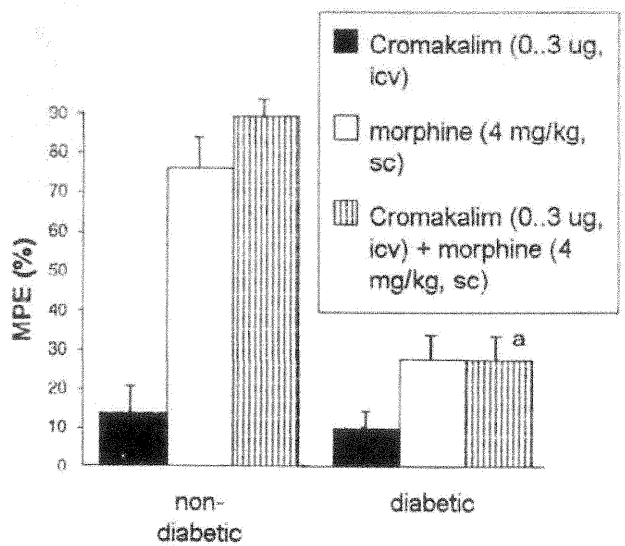

Fig. 3—Effect of cromakalim morphine and combination of cromakalim and morphine on percent MPE (mean ±SE, n=6) in non-diabetic and diabetic mice. [a=P<0.05 as compared to its non-diabetic counterpart. (Independent Student's t-test)]

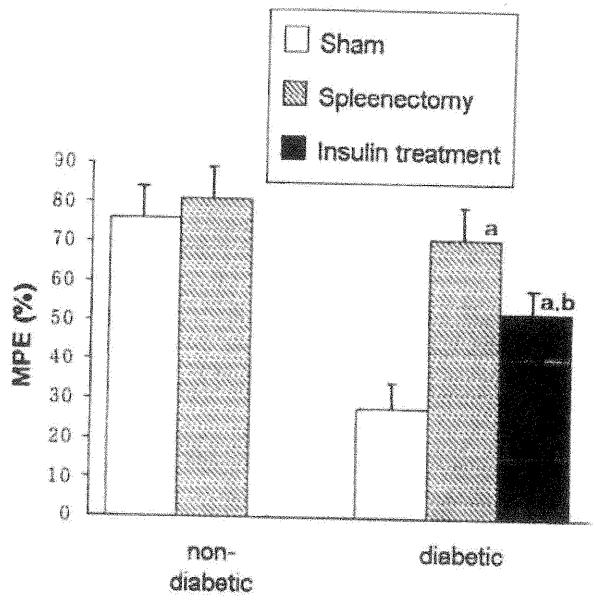

Fig. 4—Effect of spleenectomy and insulin treatment on percent MPE (mean ±SE, n=6) of morphine (4 mg/kg) in non-diabetic and diabetic mice. [a=P<0.05 as compared to sham operated diabetic control; b=P<0.05 as compared to sham operated non-diabetic control. (ANOVA followed by studentised range test, F=14.89)].

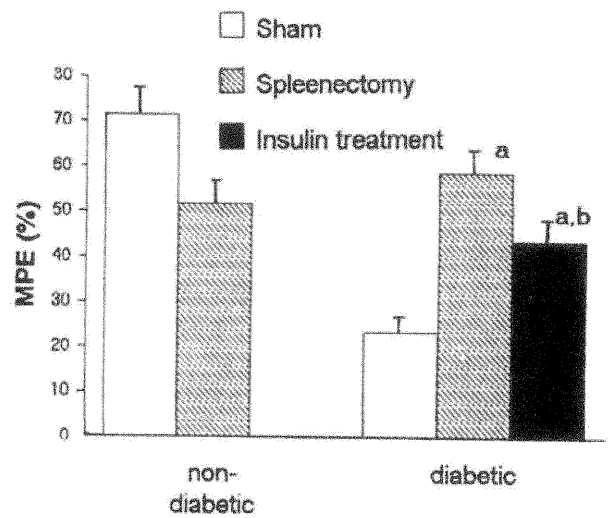

Fig. 5—Effect of spleenectomy and insulin treatment on percent MPE (mean ±SE, n=6) effect of cromakalim (2 µg iev) in non-diabetic and diabetic mice. [a=P<0.05 as compared to sham operated diabetic control; b=P<0.05 as compared to sham operated non-diabetic control. (ANOVA followed by studentised range test, F=15.25)].

Morphine produced a dose dependent relaxation of KCl precontracted ileum. pD_2 of morphine using euglycaemic (5.5 mM glucose) Tyrode's buffer was 5.32 and hyperglycaemic Tyrode's buffer (15 mM glucose) was 5.29. Therefore, the noted pD_2 of morphine was comparable.

Discussion

The results of the present study demonstrating a significant decrease in the antinociceptive effect of morphine in diabetic mice are in accordance with the earlier reports[4,16]. It is probable that increased blood glucose in diabetic mice may interfere with the binding of morphine to µ opioid receptors. pD_2 of morphine using guinea pig ileum exposed to hyperglycaemic Tyrode's buffer did not change significantly. Therefore, it may be suggested that hyperglycaemia does not interfere with the binding of morphine with µ receptors *in vitro*.

Glibenclamide, a K_{ATP} channel blocker[17], is reported to inhibit the analgesic effect of morphine[10]. K_{ATP} channel openers[18], cromakalim and pinacidil, potentiate the analgesic response of morphine[19,20]. Therefore, analgesic effect of morphine may be mediated through µ receptors[21] that are coupled to K_{ATP} channels in the central nervous system[7]. Cromakalim only slightly increased the analgesic effect of morphine in non-diabetic mice. This discrepancy with earlier reports[19,20] may be ascribed to the high doses of morphine employed in the present study which may have opened a large population of K_{ATP} channels and perhaps cromakalim has no further scope to enhance significantly the analgesic effect of morphine. However, the analgesic effect of cromakalim and morphine is still markedly reduced in diabetic mice. It tentatively suggests dysfunctioning of K_{ATP} channels as a consequence of persistent hyperglycaemia which may be responsible for noted decrease in antinociceptive effect of morphine and cromakalim in diabetic mice. Moreover, co-administration of cromakalim with morphine did not alter the reduced analgesic effect of morphine in diabetic mice. It supports our contention that dysfunctioning of K_{ATP} channels due to hyperglycaemia may be responsible for the reduced analgesic effect of morphine.

Spleen is reported to modulate analgesic effect of morphine in beige J mice[22]. Therefore, the noted decrease in antinociceptive effect of morphine in diabetic mice may be due to a factor or substance released from spleen. The noted regain in the antinociceptive effect of morphine in spleenectomised diabetic mice supports this contention. Moreover, spleenectomy-induced recovery in analgesic effect of cromakalim may tentatively suggest the involvement of spleen derived factor in dysfunctioning of K_{ATP} channels in diabetic mice. Persistent hyperglycaemia

may act as stimulus to release this unknown factor from spleen. Maintenance of euglycaemia for sufficiently long period with multiple administration of insulin, as designed in the present study, restored the analgesic response to morphine and cromakalim possibly by interfering with the hyperglycaemia induced release of this unknown factor from spleen.

On the basis of above discussion, it may be concluded that hyperglycaemia induced decrease in antinociceptive effect of morphine and cromakalim may be due to alteration in K_{ATP} channels. Some unknown factor from spleen in diabetic mice may be responsible for this alteration in K_{ATP} channels.

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