NOCICEPTION, ANTINOCICEPTIVE POTENCY OF MORPHINE IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract : There are controversial reports on the effect of diabetes on the pain threshold. We used male Wistar rats to see the effect of streptozotocin induced diabetes on the tail flick, vocalisation and vocalisation after discharge responses. These represent the spinal, lower brain stem and hypothalamic responses respectively. The effect of morphine in these parameters was studied for both the control and diabetic group. In diabetic rats, the pain threshold was increased. However, this increase was not significant. Morphine produced significant analgesia after thirty minutes for tail flick and vocalisation responses and after fifteen minutes for after discharge in the control group. The antinociceptive effect of morphine was delayed and reduced for all three pain threshold confirming the antagonistic action of glucose on opiate receptors.

Key words :   STZ streptozotocin   diabetes   morphine analgesia

INTRODUCTION

In diabetic rats the pain threshold is altered. Hyperalgesia is generally reported (1-4). However, analgesia (5-6) and no change in pain threshold has been observed (7-8). Morphine induces potent antinociceptive effect in neuropathy (9-10). The action may involve interaction with various opiod receptors. Radioligand binding and auto-radiographical studies have suggested the presence of μ, d, σ and k receptors within the central nervous system.

The antinociceptive potency of morphine is decreased in diabetes (6,7,11,12). However, little information is available about the mechanism responsible for the same. It could involve a dysfunction of the μ receptor. It has been shown that STZ induced mice are selectively hyporesponsive to supraspinal μ opiod receptor mediated antinociception but are normally responsive to activation of d and k opiod receptor (12). No differences in μ opiod receptor binding sites between diabetic and control mice are seen. Hence it may involve the μ opiod receptor coupling mechanism (13).

Dihydroetorphine produces an antinociceptive effect through activation of both μ1+μ 2 opiod receptor in mice. The diabetic mice may be hyporesponsive to supraspinal μ, opiod receptor mediated antinociception. The selective blockage of μ receptor may cause activation of a back up analgesic system. In diabetic mice naloxone induced analgesia is blocked by d opiod receptor antagonist. (14)

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Chronic pretreatment with naloxone has been known to increase tail flick latency. This attenuated the analgesic effect of δ agonist in diabetic mice, and not in non diabetic mice suggesting that this paradoxical analgesia in diabetes, is mediated via δ opiod receptors (15).

METHODS

20 male albino Wistar rats weighing 225–250 gms were used. Each was placed in a separate cage in a well ventilated room. Water and food pellets were given *ad libitum*. They were divided into two groups of ten each.

Control group

Ten rats were tested for the pain threshold. Two steel electrodes were placed in the middle of the tail (16). They were connected to a stimulator, which was indigenously designed. It worked with a frequency of 100 sec., train of 1 sec and pulse width 1.5 m/s and voltage range 0–6.2 volts. Voltage could be changed by a minimum of 0.01 volts which was displaced on the screen of the instrument.

Electrical stimulation was given and the voltage was progressively increased by 0.01 voltage till the threshold of tail flick, vocalisation and vocalisation after discharge were noted. An average of three readings at 15 min interval represented the base line value.

After a gap of 3 days the analgesic effect of morphine injected subcutaneously in dose of 5 mg/kg, body weight was tested. Pain threshold was measured before and at interval of 15 min each after injection of morphine for a period of 1 hr.

Ten rats were taken whose pain threshold was measured as with the control group. They were then injected STZ (50 mg/kg body weight) intraperiotoneally. STZ (Sigma Chemicals) was prepared in dose of 25 mg/ml and buffered with citrate at pH–4.5. Pain threshold was measured for tail flick, vocalisation and after discharge on the seventh day of injection of STZ. These represented the diabetic value.

After a gap to three days the analgesic property of morphine was tested as for the control group. Blood was taken for blood glucose estimation before the rats were sacrificed in both groups.

RESULTS

The baseline values of the control group and diabetic group were comparable (Table I). Before STZ was injected, all parameters were tested. The effect of injection of STZ was seen (Table II). The values showed slight increase in pain threshold. However, all readings were not significant (P>0.05). Morphine did not produce significant analgesia in 15 min for the tail flick threshold in the control group. In 30 min and thereafter the analgesic effect was significant. In the diabetic group significant

TABLE I : Comparison between baseline pain threshold value (in volts) of the two groups.

	Control	*Before diabetes*
	(N=10)	(N=10)
	Mean ± SD	Mean ± SD
TF	0.415 ± 0.14	0.40 ± 0.15
V	0.795 ± 0.40	0.77 ± 0.28
AD	1.890 ± 0.76	1.76 ± 1.01

All comparisons not significant (P>0.05) ;
TF : Tail flick;
V : Vocalisation;
AD : Vocalisation after discharge

analgesia was only observed at the 60 min interval. (Fig 1).

TABLE II : Effect of diabetes on the pain threshold.

Before induction of diabetes	Diabetes
Mean±SD (Volts)	Mean±SD (Volts)
TF 0.399 ± 0.15	0.433 ± 0.15
V 0.768 ± 0.29	0.896 ± 0.65
AD 1.756 ± 1.013	2.746 ± 1.929

All comparisons not significant (P>0.05) ;
TF : Tail flick;
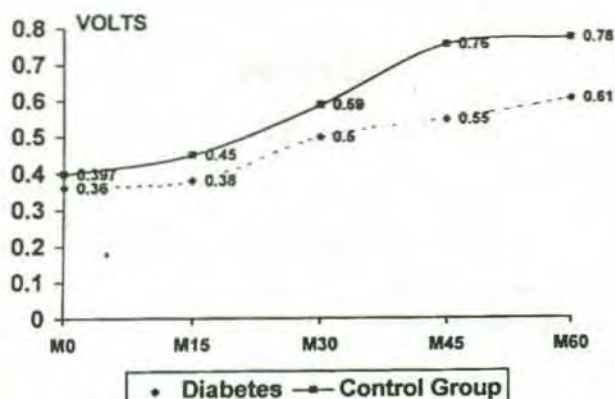V : Vocalisation;
AD : Vocalisation after discharge

Fig. 1:   Effect of morphine on tail flick in control and STZ induced diabetic group. Mo indicates before morphine administration M15 M30 M45 M60 denote at 15,30,45,60 min after morphine administration.

Values were significant at M30 in control group, at M60 in diabetic group (P<0.05)

For the vocalisation threshold significant analgesia was achieved at the 30 minutes interval for both the control and diabetic group. However, the threshold was less for the diabetic group (Fig.2).

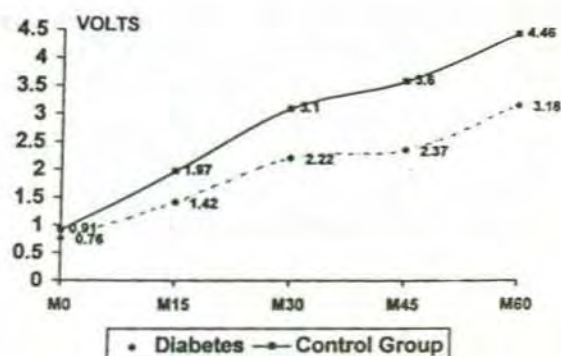The analgesic property of morphine was significant for the after discharge after 15

Fig. 2: Effect of morphine on vocalisation in control and STZ induced diabetic group.

Mo before morphine administration

M15 M30 M45 M60 - 15, 30, 45, 60 min after morphine administration.

Values significant at M30 in control group and diabetic group (P<0.05)

min in the control group. It increased with the passage of time till a period of 1 hr. This analgesia was delayed and observed after 45 min in the diabetic group (Fig. 3).
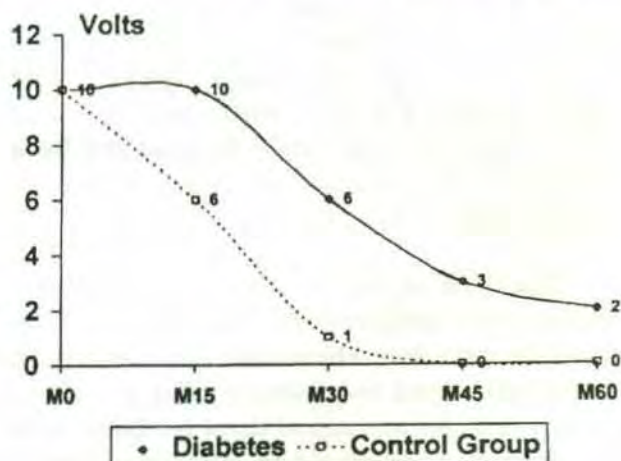

Fig. 3:   Effect of morphine on after discharge in control and STZ induced diabetic rats.

Mo- before morphine administration.

M15, M30, M45, M60 - 15, 30, 45, 60 min after morphine administration Y axis denotes number of rats pain with pain threshold n<6.2 volt. group. Significant at 15 min in control group 45 minutes in diabetic group (P<0.05).

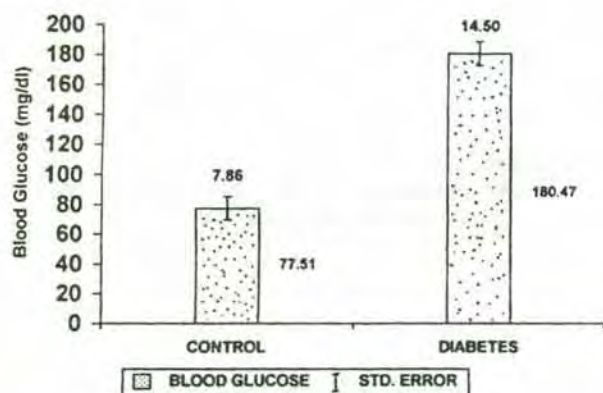The blood glucose levels in the diabetic group was significantly more than the control group (Fig. 4).

Fig. 4: Blood glucose levels in control and STZ induced diabetic groups. (Values are significant P<0.05).

DISCUSSION

Diabetes has been known to produce hyperalgesia and a painful neuropathy. We saw the effect of diabetes in all the three parameters, pain threshold in tail flick, vocalisation, and vocalisation after discharge. In all cases the threshold was increased, but the change observed was not significant. An increase in the pain threshold (5,6) and no change in threshold has also been found earlier (7,8). It is proposed that the pain threshold probably maintained because of compensatory increase in the level of endogenous opiods.

The studies confirm the analgesic property of morphine. In the control group analgesia for tail flick, vocalisation was observed after 30 min, whereas for after discharge after 15 min. In all cases the analgesic effect increased with the passage of time. The after discharge response was blocked earlier compared to the other responses. This correlates with the other study which has shown that vocalisation after discharge is blocked by lower doses of morphine compared to vocalisation during stimulation (17). The action of morphine at the nucleus ventralis posterolateralis, the somesthetic relay nucleus located in the thalamus could probably explain the effect.

The antinociceptive property of morphine is reduced in diabetic animals (6). Although morphine produced analgesia in diabetic rats, the analgesic effect was less and delayed, 30 minutes for tailflick in control compared to 60 min in diabetic rats. For vocalisation, the analgesic effect was obtained in 30 min in both group, but the effect of morphine was less in diabetic group. Similarly for the after discharge, analgesia was delayed in diabetic group. The analgesic property of morphine in blunted by acute and chronic stages of diabetes (7, 11). This effect is reversed by giving insulin. The absorption, distribution or elimination of morphine is not altered (18). It is thus suggested that the decreased antinociceptive effect of morphine in diabetes could be due to a decrease in the opiod receptor affinity or a change in their configuration (13). In conclusion our studies suggest that there is no significant change in pain threshold 1 week after induction of diabetes by STZ and that the analgesic effect of morphine is reduced in diabetes.

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