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Dopaminergic system modulation of nociceptive response in long-term diabetic rats

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Abstract

The present study examines the effects of dopaminergic system modulation on nociceptive response time in male diabetic rats. In this study, diabetes was induced by streptozotocin (STZ, 45 mg/kg) in adult male Sprague–Dawley rats. Insulin replacement therapy was initiated 6 weeks after the induction of diabetes for one-half of the diabetic group (1.5–2.5 IU/12 h/rat) and was continued throughout the duration of the study (up to 14 weeks). After 6 weeks of daily insulin replacement therapy, eight rats from each experimental group (STZ-diabetic, STZ-diabetic + insulin and nondiabetic control) were injected with either bromocriptine (BROM, 3 mg/kg/12 h), haloperidol (HALO, 1.5 mg/kg/12 h) or vehicle. Nociceptive response was measured by the hot plate (HP) latency test before the induction of diabetes (baseline), every 3 weeks for the first 12 weeks and then on days 5, 9 and 14 of treatment with dopaminergic agents. Animals were sacrificed 3 or 4 days after the last HP test and the brain, blood, spinal cord (SC), pituitary and adrenal glands (AD) were dissected for Met-enkephalin (ME) assay. The results show that nociceptive response of untreated diabetic animals increased gradually and significantly over the duration of this study. Administration of BROM and HALO significantly decreased and increased the nociceptive response, respectively, in all groups. However, the response of the diabetic group was more pronounced than that of the other two groups, especially for those treated with BROM. Daily insulin administration normalized nociceptive response to that of the nondiabetic controls. Diabetic animals receiving insulin replacement + BROM also showed normalized nociceptive response while the diabetic animals + HALO did not. Moreover, the administration of HALO and BROM resulted in an increase and decrease ME concentrations, respectively, in most tissues and brain regions examined. The effect of these dopaminergic agents on ME levels was greater in brain regions and tissues of the diabetic rats than in the diabetic groups receiving vehicle or in the nondiabetic control receiving these two agents. These data suggest that diabetes alters the sensitivity of the dopaminergic receptors and that altered response of the dopaminergic system could be indirectly involved in the modulation of nociception in diabetic rats possibly through the enhancement and/or deactivation of the endogenous Met-enkephalinergic system.

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Keywords: Nociceptive response; Diabetes; Rat; Haloperidol; Bromocriptine; Met-enkephalin

1. Introduction

Diabetes mellitus is a chronic systemic metabolic disorder manifested by serious pathological conditions including alteration in pain sensation (nociception). The etiology of the altered nociceptive response is yet to be fully determined. Data relating to the effects of experimentally induced diabetes on pain perception are inconsistent. For example, elevated pain thresholds, as measured by the hot plate (HP) and/or tail flick latency tests, have been reported in strepotozotocin (STZ)-diabetic male rats or mice (Akunne and Soliman, 1987; Chu et al., 1986; Kolta et al., 1996; Simon et al., 1981). Similarly, Morley et al. (1984) reported that diabetic humans have a lower pain tolerance to electrical stimulation when compared with nondiabetic controls. On the other hand, others researchers have shown a significant reduction in the pain threshold of STZ-diabetic female rats as measured by the HP test at 4 and 8 weeks after the induction of diabetes (Forman et al., 1986). Such differences in the reported data may be attributed to many

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factors including differences among animal model, strain, sex, age, duration and severity of the diabetic state as well as the testing method employed.

Altered nociceptive response in diabetic animal models and in humans may be explained, in part, by changes in one or more of the endogenous components that regulate nociception and/or their receptors. Several studies have demonstrated that diabetic animals are significantly less sensitive to the analgesic potency of opiate agonists. It has been shown that the antinociceptive potency of morphine and phenazocine, was significantly decreased in STZ-diabetic and spontaneously diabetic mice at 6 days after the induction of diabetes (Simon and Dewey, 1981; Simon et al., 1981). Shook and Dewey (1986) also reported that the development of physical dependence on morphine is reduced in experimental models of diabetes, suggesting alteration in the opioid receptors in these animals. The involvement of the endogenous opioid peptide systems in this mechanism also was proposed, since the administration of naloxone (Akunne and Soliman, 1987) or naltrexone (Kolta et al., 1996) reversed antinociception in STZ-diabetic rats.

On the other hand, a reciprocal relationship between dopamine (DA) and the opioid peptide ME has been well documented. It has been shown that ME levels in the striatum (STR) and neurointermediate lobe (NIL) of the pituitary were enhanced following daily administration of the DA receptor antagonist haloperidol (HALO) (Blanc et al., 1985; George and Kertesz, 1987; Llorens-Cortes et al., 1991; Sabol et al., 1983; Tang et al., 1983). In contrast, ME levels were significantly decreased in response to the DA agonist bromocriptine (BROM) (George and Kertesz, 1986). Thus, the present investigation was designed to examine such relationships in diabetes. We describe in this report the effect of pharmacological modulation of dopaminergic receptors through repeated administration of these dopaminergic agents on nociceptive response latency measured by the HP testing in long-term diabetic rats with or without insulin replacement therapy as. In addition, we also examined the changes in ME concentrations in various brain regions, spinal cord (SC), both lobes of the pituitary gland, adrenal gland (AD) as well as in plasma in response to repeated administration of these two dopaminergic agents.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) weighing 225–250 g at the beginning of the study were used. Rats were kept in a controlled environment of 21 ± 1 °C with a 12:12-h light/dark cycle (lights on at 0700 h). Food (Purina Rat Chow, Purina, St. Louis, MO) and water were provided ad libitum to all groups throughout the experimental period. Animals were maintained under these conditions for at least 1 week before experimentation. All experiments performed in this study were conducted in accordance to the "Guide for Care and Use of Laboratory Animals" provided by the National Institute of Health and adopted by Florida A&M University Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental design and drug treatment

Seventy-two animals were initially divided into two groups of control ($n = 24$) and streptozotocin (STZ) ($n = 48$). Diabetes was induced by a single intravenous injection (via the tail vein) of STZ (Sigma, St. Louis, MO), 45 mg/kg dissolved in 0.1 N citric acid buffer (pH 4.5). Control rats were injected intravenously with an equivalent volume of citric acid buffer. Three days post-injection with STZ or buffer, one drop of blood was collected from the tail vein and blood glucose levels were determined by the Accu-Check Easy Glucose monitor (Boehringer Mannheim, Indianapolis, IN) to confirm the diabetic state. Animals with blood glucose levels of greater than 300 mg/dl were considered diabetic and were used in this study. Beginning 6 weeks after the induction of diabetes, one-half of the diabetic rats ($n = 24$) were given insulin replacement therapy administered twice a day, at 0730 and 1930 h for the remainder of the experimental period (up to 56 days). Insulin (Ilentin, Eli Lilly, Indianapolis, IN) was diluted with saline solution and was administered at a dose of 1.5–2.5 IU/12 h/rat sc, based on the blood glucose level for each animal. Thereafter, insulin dose was adjusted individually every 2–3 days to maintain a glucose level comparable to that of control rats. The remaining diabetic ($n = 24$) and nondiabetic ($n = 24$) rats were injected at the same time (twice daily, sc) with an equivalent volume of saline solution. Throughout this study, blood glucose measurements were made every 2–3 days at the same time (between 1300 and 1400 h) and not on the days when the animals were subjected to HP testing. Thus, blood sampling for glucose analysis and the adjusted insulin dose for each animal were made on alternate days with HP latency testing. After 6 weeks of insulin replacement therapy, eight rats from each of the three experimental groups (STZ-diabetic, STZ-diabetic + insulin and nondiabetic control) were treated with either HALO (l.5 mg/kg sc, Sigma), BROM (3 mg/kg sc, Sigma) or vehicle (VEH, 0.3 M tartaric acid/ethanol, 3:1 v:v., sc), twice daily for 14 consecutive days. These two dopaminergic agents and vehicle were injected at the same time as insulin injections in order to minimize handling stress to the animals.

2.3. Test for nociceptive response

The time course for changes in nociceptive response was determined by the HP latency test (Hot Plate Analgesia meter, Model 39D, Columbus Instruments International,

Table 1
Body weight (g) and plasma glucose levels (mg/dl) in STZ-diabetic, STZ-diabetic treated with insulin (INS) and nondiabetic control rats

	Control (*n*)	STZ (*n*)	STZ + INS (*n*)
Initial bodyweight (g)	358 ± 4 (24)	357 ± 4 (48)	–
Bodyweight (g), 6 weeks after STZ	467 ± 7 (24)	**323 ± 10 (48)** *	–
Bodyweight (g), 3 weeks of insulin	500 ± 10 (24)	**307 ± 2 (24)** *	**451 ± 13 (24)** *
Bodyweight (g), 6 weeks of insulin	518 ± 9 (24)	**337 ± 12 (24)** *	**449 ± 12 (24)** *
Blood glucose (mg/dl), 3 days after STZ administration	107.1 ± 2.8 (24)	**480.4 ± 5.4 (24)** *	117.7 ± 6.4 (24)

Diabetes was induced by STZ (45 mg/kg, iv) dissolved in 0.1 N citric acid solution (pH 4.5). Insulin replacement (1.5–2.5 IU/12 h/rat sc) was initiated 6 weeks after the induction of diabetes and continued for six more weeks and throughout the administration of the dopaminergic agents (a total of 8 weeks). Each value represents the mean ± S.E.M. Numbers in parentheses represent the number of animals per group.

* Significant differences (in **bold**) at $P \leq .001$ from corresponding control.

Columbus, OH). Animals were placed inside a plexiglass box (30 × 30 × 45 cm), on top of a heated metal surface which was maintained at 53 ± 1 °C. The test for each animal was terminated when rats exhibited either licking of a hind paw or attempting to jump out of the plexiglass cage and the time to reach this endpoint was recorded. A cutoff time of 45 s was used to prevent injury to the animals' paws. The test was repeated every 4 min for eight consecutive sessions over a 28-min period. This protocol has been used previously in our laboratory (Kolta et al., 1996) after modification (Sahley and Berntson, 1979) and displayed any changes in the HP latency profile over a 28-min period rather than measuring a limited number of time-points which may reflect artifactual nociceptive response secondary to rapid recovery following handling stress. The initial nociceptive response measurement (baseline) for all groups was conducted 3 days before the induction of diabetes. HP tests were then conducted at the same time (between 1230 and 1600 h) on the day of testing every 3 weeks during the first 12 weeks of diabetes, as well as on days 5, 9 and 14 of treatment with the dopaminergic agents. Thus, the response to HP was always measured about 5.5 h after the morning administration of the dopaminergic agents or vehicle on each of the 3 days of testing (days 5, 9 and 14).

2.3.1. Met-enkephalin (ME) assay

Three or four days following the last HP testing, animals were sacrificed by decapitation and the adrenal, pituitary glands as well as brains regions [hypothalamus (HYPO), striatum STR] and the T_5–L_3 segment of the SC were dissected

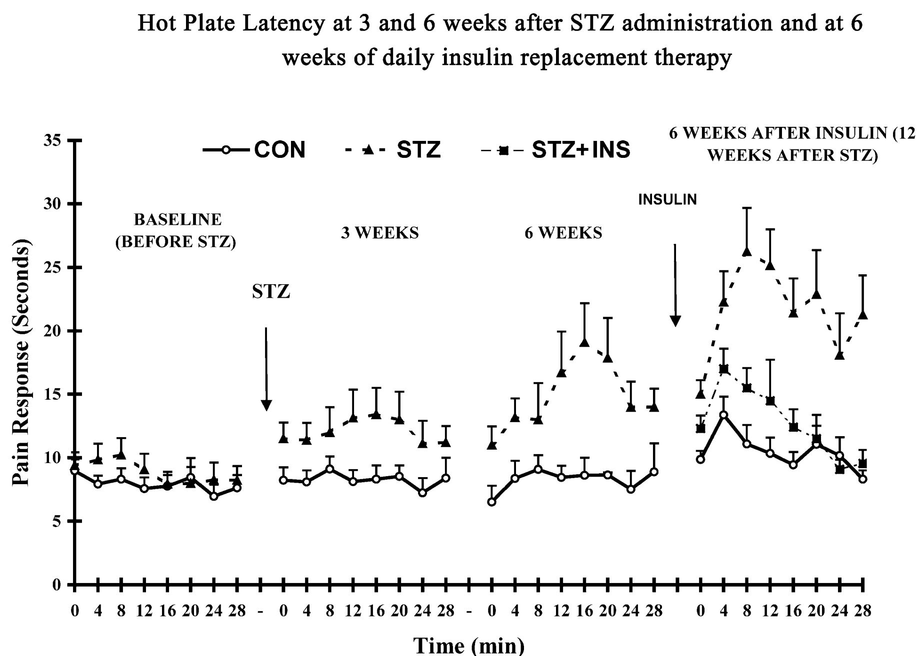

Fig. 1. Time course of the nociceptive response before the induction of diabetes, at 3 and 6 weeks after the administration of STZ (45 mg/kg, iv) and after 6 weeks of twice-daily injection of insulin (1.5–2.5 IU/12 h/rat sc). Each value is the mean ± S.E.M. (n = 24–48 rats/group).

rapidly on an ice-cold plate. The pituitary gland was further dissected into the anterior pituitary (AP) and the neurointermediate lobe (NIL). Tissues were weighed and placed immediately in ice-cold tubes containing 0.5 N HCl + 0.1% EDTA, homogenized, centrifuged at 40,000 × g for 30 min at 4 °C, then the supernatant was separated. Blood samples were also collected into plastic tubes (Sarstedt, Princeton, NJ) containing a mixture of ice-cold aprotinin, 5% EDTA and 17% citric acid, mixed well then centrifuged at 16,000 × g for 45 min at 4 °C. Plasma was separated and 100 μl of 5 N HCl was added to each 1.0 ml of plasma. All tissues, supernatant and plasma samples were stored at − 80 °C until ME assay. The ME-like immunoreactivity (ME-LI) assay was measured by the RIA method as described previously (Kolta et al., 1992) using ME standard (Peninsula Laboratories, Belmont, CA), rabbit antiserum (Immunonuclear, Stillwater, MN) and ^{125}I-ME (New England

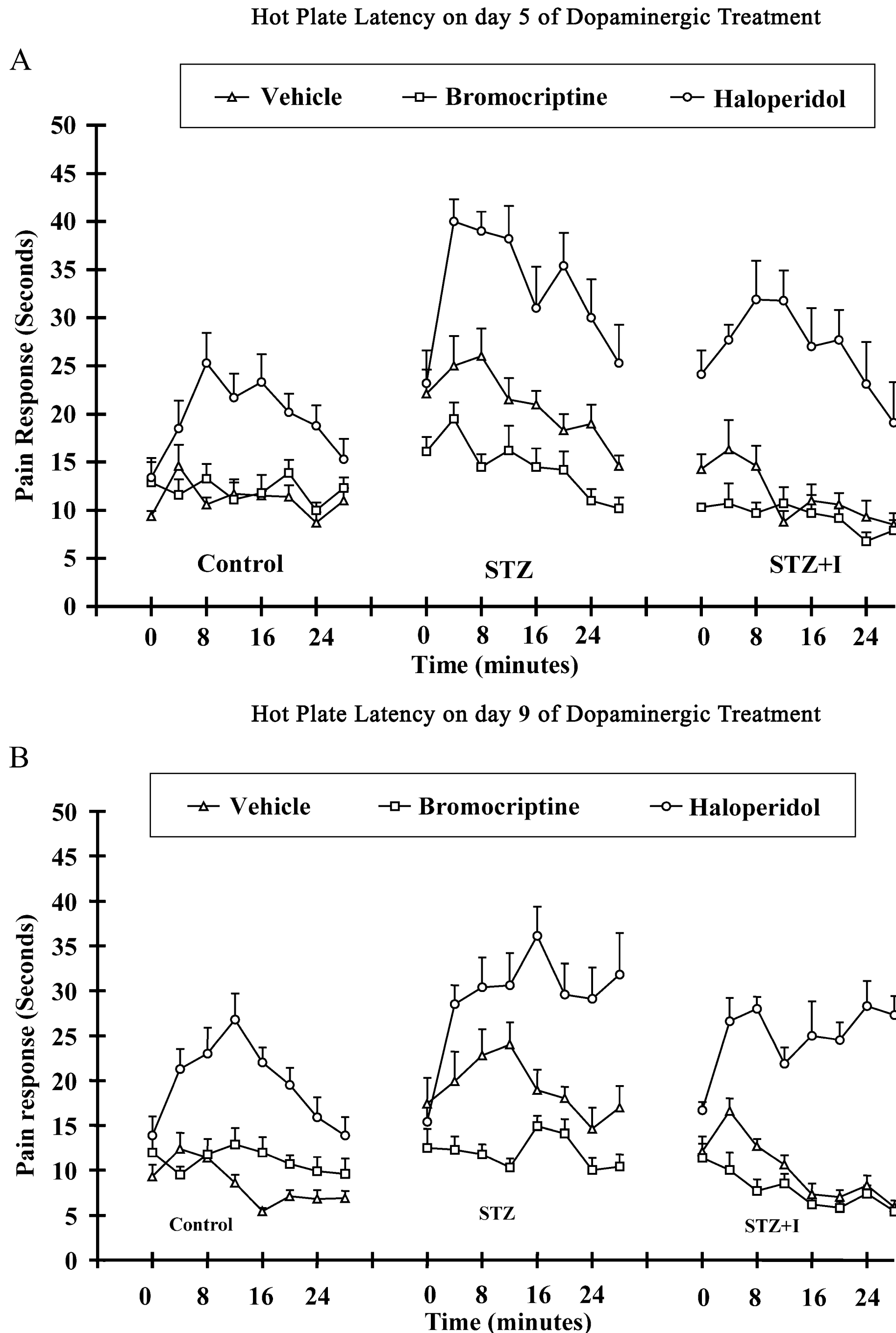

Fig. 2. (A–C) Time course of the nociceptive response of the three experimental groups on day 5 (A), day 9 (B) and day 14 (C) of daily administration of the dopaminergic agents, HALO (1.5 mg/kg, sc), BROM (3 mg/kg, sc) or vehicle to STZ-diabetic, STZ-diabetic + insulin and nondiabetic control rats. The treatment was initiated 6 weeks following insulin replacement therapy (about 12 weeks after the induction of diabetes with STZ). Each value represents the mean ± S.E.M. (n = 6–8 animals/group).

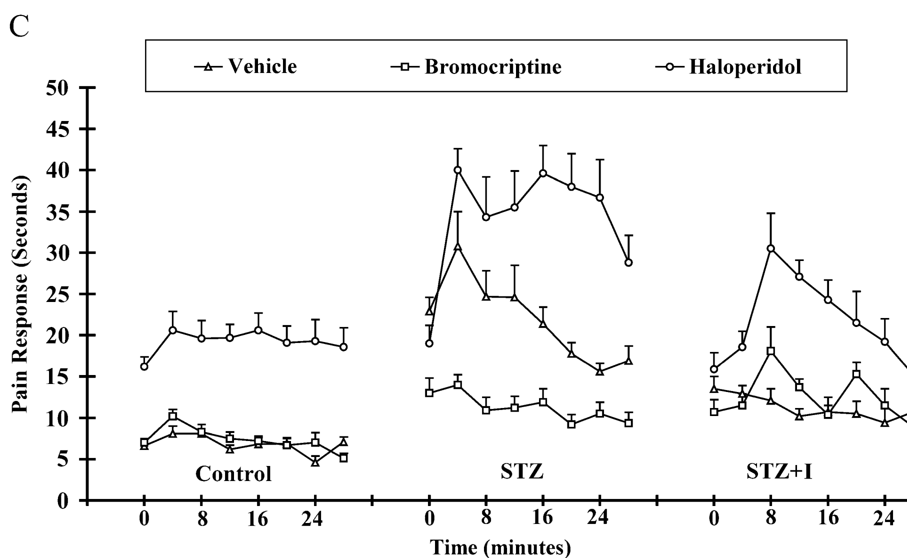

Fig. 2 (*continued*).

Nuclear, Boston, MA). The final concentration of ME in each sample was expressed as pmol/g tissue or pmol/l for the plasma.

2.4. Statistical analysis

Repeated-measures analysis of variance (ANOVA) with Bonferroni multiple comparisons test was used to determine the significance of the HP response among control, diabetic and insulin-treated diabetic rats for all drug treatments. For comparison of ME levels, differences between the means of the groups were analyzed using one-way ANOVA. The level of significance was set at $P \leq .05$ for all comparisons unless otherwise indicated.

3. Results

The effects of diabetes induced by STZ on body weight and blood glucose level are shown in Table 1. STZ-induced diabetes caused a marked decrease in body weight as expected and blood glucose concentration was significantly higher in the diabetic rats 3 days after the administration of STZ. Daily insulin replacement therapy resulted in an increase in body weight to almost that of nondiabetic animals while blood glucose returned to a level not significantly different from that of the control values.

3.1. Nociceptive response time course

The nociceptive response (s) of the experimental groups to the HP before the administration of STZ (baseline) and at 3 and 6 weeks after the induction of diabetes is presented in Fig. 1. Three weeks after STZ administration, the response of the diabetic rats to the HP was significantly longer ($P \leq .001$) with a mean difference of 3.3 s than that of the nondiabetic controls. Six weeks postinduction of diabetes, the response to the HP by the diabetic rats was even longer than at 3 weeks with a mean difference of 7.6 s when compared with saline controls ($P \leq .0001$). Fig. 1 also shows that 12 weeks after the induction of diabetes, the marked elevation in the nociceptive response of the diabetic animals as compared with the nondiabetic controls ($P \leq .0001$) was still present. Twice-daily injection of insulin initiated 6 weeks after the induction of diabetes normalized this response to a level which was not significantly different ($P > .05$) from that of saline-treated control animals.

3.2. Effect of dopaminergic agents on the nociceptive response

Fig. 2A–C presents the pain response data obtained during the course of treatment with the dopaminergic agonist (BROM) or antagonist (HALO) measured on days 5 (Fig. 2A), 9 (Fig. 2B) and 14 (Fig. 2C). One day before the administration of these dopaminergic agents, the baseline response for each group was established (data not shown). Fig. 2A shows that BROM administration for 5 days significantly ($P \leq .05$) attenuated the nociceptive response only in the diabetic group. The response of the other two groups (STZ + insulin and nondiabetic control) to BROM was similar to that of the same groups receiving vehicle. Fig. 2A also shows that HALO administration caused a significant elevation of the nociceptive response in the three experimental groups, but the response was greater in the diabetic rats than in the other two groups receiving the same agent. The reaction time to HP by all groups receiving HALO was significantly longer than the response of the same groups receiving either vehicle

($P \leq .005$) or BROM ($P \leq .0001$). Fig. 2B shows that the nociceptive response of the three groups on day 9 of the daily treatment with dopaminergic agents was similar to that on day 5. However, the response of the diabetic rats to BROM on day 9 was attenuated even further (mean response of 11.78 ± 0.62) than the response of the same group on day 5 (mean response of 14.25 ± 0.10). Fig. 2C shows the nociceptive response measured on day 14 of treatment with the dopaminergic agents or saline. The data obtained from the three experimental groups show the same trends reported previously, but the magnitude of the response was greater than that measured on days 5 and 9. It is interesting to note that over the course of treatment, the response of the diabetic rats to BROM and HALO was more pronounced than the other two groups causing gradual and significant decrease and increase, respectively, in the nociceptive response from day 0 (the day before the administration of the dopaminergic agents) to day 9, with no further changes at day 14.

3.2.1. ME concentrations in tissues and plasma

Table 2 presents the ME concentrations in brain regions and tissues (pmol/g) and plasma (pmol/l) obtained from the three experimental groups after 14 days of daily administration of the dopaminergic agents followed by a 3- or 4-day recovery period. In the AP, HALO administration caused a significant increase ($P \leq .01$) in ME concentration of the nondiabetic control group when compared with control animals receiving vehicle. ME concentrations were also significantly decreased ($P \leq 0.05$) in the same region (AP) of the control and diabetic animals receiving BROM, but the decrease was much greater in diabetic animals as compared to the control or to the diabetic group receiving vehicle (62% vs 38%). In the NIL, HALO caused an insignificant slight increase or no changes in ME concentrations in all groups, while BROM caused a significant decrease ($P \leq .05$) in ME concentrations in the NIL of the three experimental groups ranging from 27% to 47%, when compared with the matching vehicle-treated animals. In the HYPO, HALO administration resulted in a significant increase in ME concentrations in the nondiabetic control ($P \leq .05$) as well as in the diabetic + insulin group ($P \leq .005$) when compared with the matching vehicle-treated rats. BROM administration caused a slight but significant increase ($P \leq .05$) in ME concentration only in the diabetic + insulin group. In the STR, HALO caused a significant increase ($P \leq .05$) in ME concentrations only in the control group, while treatment with BROM caused a significant decrease ($P \leq .01$) in ME concentration in the diabetic group when compared with their matching vehicle-treated groups. In the SC, HALO administration resulted in significantly increased ME concentrations ($P \leq .01$) in the diabetic animals and nondiabetic control when compared with the vehicle-treated rats. BROM administration caused a significant decrease ($P \leq .01$) in ME in the SC of the control animals only. In the AD, repeated HALO administration for 14 days resulted in a significant increase ($P \leq .01$) in ME concentrations in both the control and diabetic groups, while BROM administration caused a significant change in ME concentrations in the AD of only the nondiabetic control when compared with the vehicle-treated animals. In plasma, HALO administration resulted in a significant increase ($P \leq .05$) in ME concentration of the diabetic and diabetic + insulin rats when compared with the vehicle controls. BROM administration caused a significant decrease ($P \leq .01$) in plasma ME concentrations of the control and diabetic rats.

4. Discussion

The present study demonstrates that long-term diabetes induced by STZ in adult male rats results in a gradual elevation of the nociceptive response time as measured by the HP latency test. This hypoalgesic-like state was evident in the nontreated diabetic group before (Fig. 1) and during the administration of the dopaminergic agents (Fig. 2A, B

Table 2
Met-enkephalin concentrations (pmol/g) in adrenal and pituitary glands, brain regions and in plasma (pmol/l), 14.5 weeks after the induction of diabetes with STZ

Tissue	Control			STZ diabetic			STZ diabetic + insulin		
	VEH	HALO	BROM	VEH	HALO	BROM	VEH	HALO	BROM
AP	44 ± 5	**88 ± 3** *	**27 ± 3** *	76 ± 4	80 ± 3	**29 ± 12** *	51 ± 3	59 ± 3	37 ± 9
NIL	600 ± 40	670 ± 60	**440 ± 15** *	520 ± 75	680 ± 50	**310 ± 15** *	525 ± 80	430 ± 60	**280 ± 50** *
HYPO	687 ± 65	**950 ± 50** *	745 ± 40	1000 ± 120	950 ± 20	**740 ± 90** *	520 ± 50	**930 ± 50** *	**730 ± 35** *
STR	800 ± 109	**1200 ± 110** *	800 ± 94	1166 ± 93	905 ± 171	**670 ± 93** *	920 ± 85	810 ± 129	640 ± 100
SC	20 ± 1.5	**34 ± 5** *	**11 ± 1** *	38 ± 6	**67 ± 7** *	33 ± 5	22 ± 8	25 ± 5	20 ± 3
AD	2.7 ± 0.5	**5.5 ± 0.8** *	**4.5 ± 0.5** *	3.8 ± 0.8	**10.4 ± 0.7** *	5.4 ± 1.3	2.6 ± 0.2	2.5 ± 1	1.9 ± 0.2
Pl	22 ± 1.5	18 ± 2	**9.8 ± 1** *	42 ± 2	**69 ± 14** *	**15 ± 5** *	18 ± 2	**56 ± 13** *	22 ± 2

Diabetes was induced by STZ (45 mg/kg, iv) dissolved in 0.1 N citric acid solution (pH 4.5). Insulin replacement (1.5–2.5 IU/12 h/rat, sc) was initiated 6 weeks after the induction of diabetes. Met-enkephalin concentrations was measured by RIA in anterior pituitary (AP), neurointermediate lobe (NIL), hypothalamus (HYPO), striatum (STR), spinal cord (SC), adrenal gland (AD) and plasma (Pl), 14 days after daily administration (sc) of haloperidol (HALO, 1.5 mg/kg), bromocriptine (BROM, 3 mg/kg) or vehicle (VEH) to control, STZ-diabetic and STZ-diabetic + insulin rats. Rats were sacrificed 3 or 4 days after the last dose of dopaminergic agents. Each value is the mean ± S.E.M. ($n = 5-8$/group).

* Significant differences (in **bold**) at $P \leq .001$ from their vehicle-treated animals.

and C). Furthermore, daily insulin replacement therapy to one-half of the diabetic animals, initiated 6 weeks after the induction of diabetes with STZ and continued for an additional 6 weeks and throughout the 14 days treatment with the dopaminergic agents, normalized nociceptive response to a level that was indistinguishable from that of the nondiabetic controls (Fig. 1). These results are in agreement with previous reports from our laboratory (Kolta et al., 1996) and by others which indicated that the hyperglycemic state or STZ-induced diabetic rats (Akunne and Soliman, 1987) and spontaneously diabetic mice (Simon and Dewey, 1981) have a significantly higher nociceptive response than their respective controls. These data suggest that chronic diabetes, or hyperglycemia per se, alters the antinociception response when measured by the HP method.

Considerable evidence indicates that DA is one of the major neurotransmitters mediating nociceptive response. Several reports have described a direct role for DA in nociceptive mediation (Frussa-Filho et al., 1996; Kiritsy-Roy et al., 1989; Lin et al., 1989; Morgan and Franklin, 1991; Suandeau and Costentin, 1995), while others provided evidence for an indirect role for DA in the altered nociceptive response through its interaction with one of the endogenous opioid peptide systems known to be involved in the antinociception process (Kamei and Saitoh, 1996; Takeshita and Yamaguchi, 1998). Our study would have supported a direct effect of DA in this response, if the administration of BROM and HALO had induced an increase and decrease in response time to HP, respectively. However, the results obtained from the present investigation regarding the effect of repeated exposure to BROM and HALO on nociceptive response support an indirect role for DA. We demonstrated in the present study that twice-daily administration of the dopaminergic agonist (BROM) or antagonist (HALO) for 14 consecutive days caused a gradual attenuation and potentiation of nociceptive response, respectively, in all three experimental groups, implicating a role for the dopaminergic receptors in the reaction to such noxious stimuli. However, the response of the STZ-diabetic animals to these two agents was much greater than that of the other two groups, especially to BROM and, to a lesser extent, to HALO. These results suggest that the diabetic state alters the normal response to dopaminergic agents, becoming more sensitive to these agents. Moreover, daily insulin administration to the diabetic animals normalized nociceptive response to BROM. Thus, it seems that the significant attenuation of the nociceptive response induced by BROM in the diabetic group, but not in the other two experimental groups (diabetic + insulin and nondiabetic control), is due to the fact that these two groups have already achieved their lowest attainable reaction time to HP. On the other hand, diabetic animals with high nociceptive latency that progresses as the diabetic state become chronic, retain some capacity for attenuation as induced by this dopaminergic agonist.

The discrepancy in the role of DA mediation of the nociceptive response may be the result of differences between experimental designs. One of the main differences between this study and previously reported studies was in the drug administration regimen of HALO and BROM (dose, frequency and duration). In this study, we used repeated doses (twice a day) for 14 consecutive days (closely similar to that reported previously by George and Kertesz, 1986, 1987) as opposed to a single dose reported by many. Another important factor is the duration of diabetes in our study where rats were diabetic for 12 weeks prior to receiving these dopaminergic agents (a total duration of at least 14 weeks of diabetes). Therefore, it seems possible that the effect of the dopaminergic agents on nociceptive response latency reported here is an indirect effect mediated mainly through interaction with another endogenous neurotransmitter or modulator such as ME. This endogenous pentapeptide, as well as the availability of the δ- and μ-opioid receptors, has been shown to play a major role in nociceptive mediation in diabetic animal models (Coudore-Civiale et al., 2001; Kamei et al., 1997, 2000).

Several lines of evidence provided information regarding a functional link (other than locomotor activity and stereotyped behavioral) between the DA and opioid systems. Kamei and Saitoh (1996) reported that sulpiride or quinpirole enhances morphine-induced antinociception in short-term (2 weeks) diabetic mice in a dose-dependent manner but not in nondiabetic controls. Thus, it is possible that this effect of morphine in diabetic animals may be influenced by the reduction in DA transmission through D_2 receptor blockade. Furthermore, interactions between DA and the opioid peptide ME in various brain regions have been well documented and were further examined in the present study in diabetic rats. We found that BROM administration decreased ME concentration in the NIL of the diabetic rats as compared to that of the same group receiving vehicle or the nondiabetic group receiving BROM. It has been shown that repeated activation of dopaminergic receptors by BROM for 9 days attenuated the levels of the endogenous ME-LI in the NIL of the pituitary (George and Kertesz, 1986). BROM administration also resulted in a small (10–20%) decrease in striatal ME-LI levels (George and Kertesz, 1986). Conversely, DA receptor blockade with HALO increased striatal ME concentrations by about 50% (George and Kertesz, 1987). The effect of HALO on striatal ME levels has been shown to be secondary to an increase in ME biosynthesis, accelerated precursor processing and small increases in degradation (Blanc et al., 1985; Hong et al., 1979; Mocchetti et al., 1985). Similarly, depletion of presynaptic DA (with reserpine) resulted in a marked (50–60%) augmentation of ME levels for up to 20 days thereafter (Tang, 1991). In addition, interruption of dopaminergic transmission enhanced the steady-state levels of enkephalin or proenkephalin and proenkephalin mRNA abundance (Morris et al., 1988; Sivam et al., 1986; Tang et al., 1983). The present study also shows that HALO administration increased ME concentrations in the SC, AD and plasma of the diabetic groups as compared to that of the same groups receiving vehicle or the nondiabetic control receiving

this dopaminergic antagonist. The increase in ME concentration in these tissues of the diabetic rats in response to HALO could resulted from enhancement in the release of ME and/or decrease in ME turnover rate. These data support a reciprocal effect for dopaminergic influence on ME, which seems to be exacerbated in diabetes. This peptide has been shown to induce analgesia (Hughes et al., 1975; Kosterlitz and Hughes, 1975) and to increase in various brain regions and tissues in diabetic animals (Kolta et al., 1992, 1996). Further support for the involvement of both ME and DA systems in the altered nociceptive response in diabetic rats is provided by the data obtained from the present study, where we found that in nondiabetic animals the time to react to HP was increased and decreased in response to repeated HALO and BROM administration, respectively. The elevated HP response in the control group was accompanied by a significant increase in ME concentrations in most tissues examined (AP, HYPO, STR, SC and AD), while the attenuated nociceptive response was accompanied by a significant decrease in ME concentrations in several tissues (AP, NIL, SC and plasma). Furthermore, we reported previously (Kolta et al., 1996) that blocking or direct deactivation of ME receptors by a small dose of naltrexone (0.3 mg/kg) significantly attenuated the pain threshold response to HP and tail flick in chronically diabetic male rats. Together, these data strongly support the hypothesis that the altered nociceptive response in diabetic animals results from indirect dopaminergic influence on the opioid peptide system ME.

In conclusion, the present results suggest a reciprocal relationship between the dopaminergic and ME systems in modulating the altered nociceptive response in diabetes. The exact mechanism by which the dopaminergic agents employed in the present study affected the response to HP, is yet to be fully determined. However, considerable data in the literature and our present results implicate one of the opioid peptide systems (ME) in such a response, but do not exclude the involvement of other systems.

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