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Antinociceptive Effects of Morphine Were Different Between Experimental and Genetic Diabetes

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TAKESHITA, N. AND YAMAGUCHI, I. *Antinociceptive effects of morphine were different between experimental and genetic diabetes.* PHARMACOL BIOCHEM BEHAV **60**(4) 889–897, 1998.—This study was designed to investigate the effect of morphine on formalin-induced nociceptive responses in streptozotocin (STZ) induced-diabetic mice, noninsulin-dependent genetically diabetic db/db mice and their respective controls (ddY and +/+). In nondiabetic (ddY and +/+) mice, morphine (1–10 mg/kg, PO) dose dependently attenuated the biphasic nociceptive responses induced by SC injection of formalin to the hindpaw, demonstrating equipotency on both the first and second phases. Para-chlorophenylalanine (800 mg/kg × 2, PO) and pindolol (1 mg/kg, IP) reduced the effect of morphine on the first phase, sulpiride (10 mg/kg, IP) abolished the effect on both phases, while ketanserin (1 mg/kg, IP) had no effect. In STZ (200 mg/kg, IP)-diabetic mice, morphine weakly attenuated the nociception in comparison to control ddY mice, whereas it had comparable effects in both the first and second phases of control +/+ mice and db/db mice. The serotonergic agonist, meta-chlorophenylpiperazine (0.32–3.2 mg/kg, PO), dose dependently attenuated the biphasic nociceptive responses to formalin in both phases of diabetic mice; however, FR64822, a dopaminergic compound (0.1–10 mg/kg, PO), had little effect. We speculate that activation of both dopaminergic (DA)- and serotonergic-mediated mechanisms are potentially responsible for the effect of morphine on the first phase, while the DA-mediated effect is involved in the second phase. The DA-mediated mechanism, but not the serotonin-mediated one, appears to be altered in both STZ-diabetic and db/db mice. These results suggest that the attenuated effects of morphine might be due to a dopaminergic dysfunction in STZ mice, and that there might be other mechanisms compensating for this attenuation of dopaminergic function in db/db mice. © 1998 Elsevier Science Inc.

Morphine Formalin test Antinociception 5-Hydroxytryptamine Dopamine Diabetic mice

IT has been reported that streptozotocin (STZ)-induced diabetic mice and rats (insulin-dependent diabetic model) and genetically diabetic db/db mice (noninsulin-dependent diabetic model) are significantly less responsive to the antinociceptive effect of morphine in the tail-flick test (30). These authors postulated that hyperglycemia might interfere with morphine action on the opioid receptor. In support of this hypothesis, Brase et al. demonstrated that glucose caused small but significant concentration-dependent decreases in opiate receptor affinities for [^{3}H]naloxone and [^{3}H]dihydromorphine (1). However, the binding affinities were similar in db/db, STZ-treated, and control mice (1). Therefore, it is clear that further studies are needed to clarify the mechanism responsible for the reduced effectiveness of morphine in diabetic animals.

Opioid receptors associated with analgesia have been subdivided into mu-, delta-, and kappa-receptors, with morphine considered to selectively activate spinal and supraspinal mu-opioid receptors. Given that in comparison with control mice STZ-treated mice are hyporesponsive to intracerebroventricular, but not to intrathecal injections of morphine (15), it is possible to speculate that a mechanism mediated by supraspinal mu-opioid receptors may be altered in diabetic mice. However, stimulation of supraspinal mu-opioid receptors by morphine leads to the release of 5-hydroxytryptamine (5-HT),

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dopamine (DA), and norepinephrine (NE), which are all potentially involved in the analgesic action of morphine (5–7,14,21,22). Interestingly, several lines of evidence indicate that monoamine turnover is depressed in the brains of both humans and animals with diabetes mellitus (4,33–35).

The formalin test, an animal model known to resemble certain kinds of clinical pain, was originally described in rats and cats by Dubuisson and Dennis (9). In this model, the pain produced in rats by SC injection of formalin is biphasic: in the first phase the pain stems from direct stimulation of nerve endings, while in the second phase the pain is due to the subsequent inflammation (9,28). Another important characteristic of this test is that application of formalin results in the activation of 5-HT (31), DA (22), NE (7), and opioid (7,21,24) receptors, all of which are involved in antinociception. Thus, the formalin test is considered to be an appropriate model for studying changes in monoaminergic and opioidergic antinociceptive mechanisms. However, there are only a few reports on the involvement of both 5-HT and DA in the antinociceptive mechanism of morphine using the formalin test.

In the present study, we examined the effects of morphine on formalin-induced nociceptive responses in db/db and STZ-treated mice with short-term diabetes, and compared them with control (ddY and +/+) mice. The mechanism of antinociceptive action of morphine was also studied in ddY mice. In addition, we examined the effects of a dopaminergic and a serotonergic compound on the nociceptive response in both control and diabetic mice.

METHOD

Animals

All the mice were housed at 22 ± 1°C and 55 ± 5% humidity under a 12 L:12 D cycle and were given access to water and chow ad lib.

STZ-induced diabetic mice: 5-week-old male ddY mice (SLC, Shizuoka, Japan) were injected intraperitoneally (IP) with 200 mg/kg of STZ or 2 mM citrate buffer (pH 4.5) vehicle. Ten days later blood was taken from the orbital sinus under ether anesthesia and plasma glucose levels were determined using a commercial kit (Glucose B-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Mice with blood glucose levels of >400 mg/dl were used for testing with formalin 2 weeks after administration of STZ. Age-matched male ddY mice were used as the control for STZ-diabetic mice.

Genetically diabetic mice. Six-week-old female C57BL/KsJ-db/db mice were purchased from Jackson Labs (Bar Harbor, ME). The effect of formalin was examined when mice were 9–10 weeks old because hyperglycemia stabilized at 7–8 weeks of age. Blood was taken from the orbital sinus under ether anesthesia and plasma glucose levels were determined as described above, with db/db mice with blood glucose levels of >400 mg/dl used for testing. Age-matched nondiabetic littermates (+/+ mice) were used as the control for the formalin-induced nociceptive response.

This study was carried out in accordance with the Declaration of Helsinki and/under the guidelines established by our company for the care and use of laboratory animals.

Drugs

Morphine hydrochloride was obtained from Takeda Pharmaceutical Co. Ltd. (Osaka, Japan). (±)-Pindolol hydrochloride, *para*-chlorophenylalanine hydrochloride (PCPA), and STZ were obtained from Sigma Chemical Co. (St. Louis, MO). Ketanserin tartrate and meta-chlorophenylpiperazine (m-CPP) were from Research Biochemical Inc. (Natick, MA). Sulpiride and FR64822 (N-(4-pyridylcarbamoyl)-amino 1,2,3,6,-tetrahydropyridine) were synthesized in our laboratory. Formalin was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Morphine, PCPA, m-CPP, ketanserin, and formalin were dissolved and diluted in saline. Pindolol and sulpiride were dissolved in 1% tartaric acid, adjusted to pH 7 with 1 N NaOH and diluted in saline. STZ was dissolved in 2 mM citrate buffer at pH 4.5. FR64822 was suspended in 0.5% methylcellulose solution. PCPA (800 mg/kg) was given PO 2 and 3 days before the formalin test. Morphine, FR64822 and m-CPP were administered PO 60 min before the test. Pindolol, ketanserin, and sulpiride were given IP 30 min before morphine administration.

Formalin Test

We have previously described slight modifications (31) to the formalin test published by Hunskaar et al. (13). Mice were placed in an observation chamber 5 min prior to injection of formalin to allow for acclimatization. Ten milliliters of 1% formaldehyde in saline were administered into the left hindpaw using an Ito microsyringe (Shizuoka, Japan). Animal were then returned to the observation chamber and nociceptive responses were recorded for a period of 30 min. The summation of time (in seconds) spent licking and/or biting of the injected paw during each 5-min block was measured as an indicator of pain response. The duration of responses in the first 10 min and from 10 min to 30 min represented the first and second phases, respectively.

Statistical Analysis

The results are presented as mean ± SEM. Statistical significance of differences between groups were assessed using analysis of variance (ANOVA) followed by a Dunnetts' *t*-test or by an unpaired t-test where indicated. *p*-Values of less than 0.05 were considered significant. The ID_{50} (i.e., dose of drug that reduced formalin-induced nociception by 50% relative to control values) was estimated from individual experiments by using linear regression analyses and an in-house computer program.

RESULTS

Formalin-Induced Nociceptive Response in Normal and Diabetic Mice

Subcutaneous (SC) injection of formalin into the hindpaw of normal ddY mice resulted in licking and biting of the injected paw. The nociceptive responses were biphasic: an acute, immediate response that peaked at 5 min and disappeared within 10 min (first phase), and a second response that peaked at 15–25 min and lasted more than 30 min (second phase). In ddY mice, the total response times for the first and second phases were 99.6 ± 6.6 and 147.1 ± 12.4 s, respectively (Table 1). Although the time length of the first phase was not significantly different between STZ and ddY mice, the second phase was significantly shorter in the STZ mice. In db/db mice, total response times for both phases were significantly shorter than in control +/+ mice.

Antinociceptive Effects of Morphine in Normal and Diabetic Mice

Figure 1 shows the effect of morphine on formalin-induced nociceptive responses in both diabetic and control mice. In

TABLE 1
TOTAL NOCICEPTIVE RESPONSE TIMES INDUCED BY FORMALIN IN MICE

Mice	Times (s)	
	First Phase	Second Phase
ddY	99.6 ± 6.6	147.1 ± 12.4
STZ	83.5 ± 4.3	55.6 ± 14.1*
+/+	92.9 ± 9.6	202.8 ± 25.4
db/db	46.7 ± 6.9*	60.0 ± 11.6*

First phase and second phase represent the sum of nociceptive responses during the 0–10-min and 10–30-min time periods following formalin injection. Each value is represented as the mean ± SEM ($n = 10$), with statistical differences ($*p < 0.001$) shown against their respective controls for each phase (Student's *t*-test).

ddY mice (Fig. 1a), morphine significantly reduced the nociceptive response time of both the first, $F(4, 45) = 22.11$, $p < 0.001$, and second phases, $F(4, 45) = 6.38$, $p < 0.001$. The ID_{50} values for morphine were comparable in the first ($ID_{50} = 16.0$ mg/kg) and second ($ID_{50} = 8.9$ mg/kg) phases. The antinociceptive effectiveness of morphine was greatly reduced in the STZ-treated rats (Fig. 1b); 10 mg/kg of morphine did not significantly attenuate the formalin-induced responses in either phase. The overall dose effect of morphine was just statistically significant for the first phase, $F(4, 45) = 3.54$, $p < 0.05$, but not for the second, $F(4, 45) = 1.05$, $p > 0.05$.

In db/db mice (Fig. 1d), morphine dose dependently reduced the nociceptive response time of the first, $F(4, 40) = 22.63$, $p < 0.001$, and second, $F(4, 40) = 6.76$, $p < 0.001$, phases, with ID_{50} values of 6.4 and 5.8 mg/kg, respectively. Statistically significant differences from control were obtained at 10 and 32 mg/kg. Comparative antinociceptive activity was also observed in the nondiabetic (+/+) mice (Fig. 1c) with 10 and 32 mg/kg of morphine significantly attenuating the formalin-induced nociception. The overall dose effect of morphine was statistically significant for the first, $F(4, 40) = 112.7$, $p < 0.001$, and second phases, $F(4, 40) = 55.6$, $p < 0.001$, with ID_{50} values of 11.5 and 13.6 mg/kg for each phase, respectively.

Effects of PCPA Treatment on the Antinociceptive Activity of Morphine

Oral administration of morphine (10 mg/kg) significantly inhibited the first (Fig. 2a) and second phase (Fig. 2b) of formalin-induced nociception in normal ddY mice, with inhibition being 43 and 53%, respectively. ddY mice pretreated with PCPA (a 5-HT depleter) showed a 50% reduction in brain 5-HT levels with norepinephrine and dopamine levels unaffected (data not shown). The PCPA treatment alone did not significantly change the nociceptive responses to formalin injection (compare saline–saline with PCPA–saline); however, it greatly attenuated the effect of morphine on the first

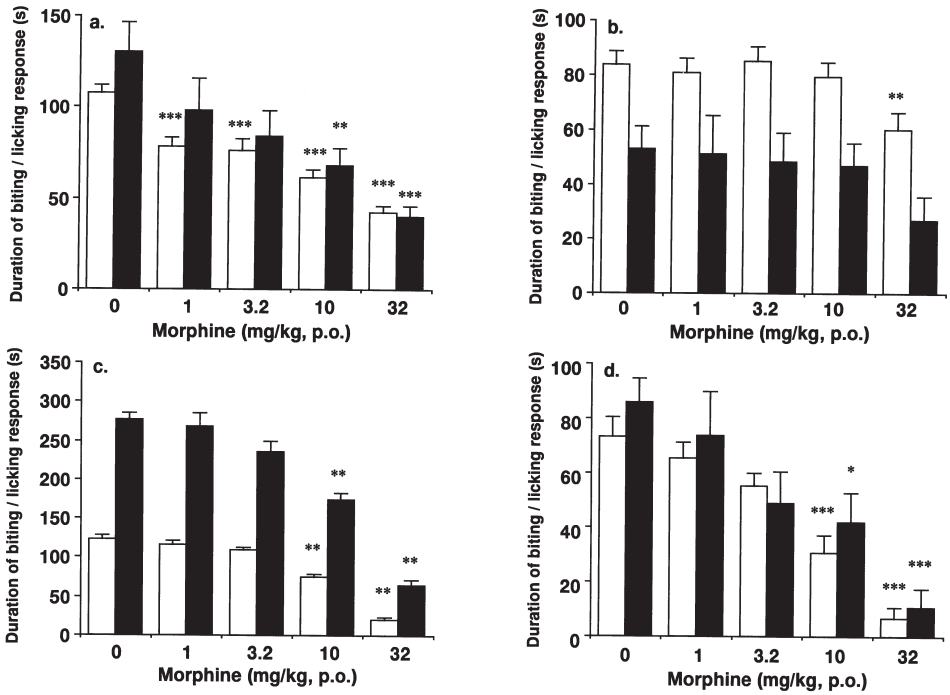

FIG. 1. Effects of morphine on formalin-induced nociceptive responses in normal ddY (a), STZ-induced diabetic (b), normoglycemic +/+ (c), and genetically diabetic db/db mice (d). Morphine was administered PO 60 min prior to formalin injection. First phase (open columns) and second phase (closed columns) represent the summation of nociceptive responses during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM (n = 9–10). Dunnetts' *t*-test for multiple comparisons subsequent to ANOVA was used for evaluation. Statistically significant values ($*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ are shown against their respective 0-mg/kg groups.

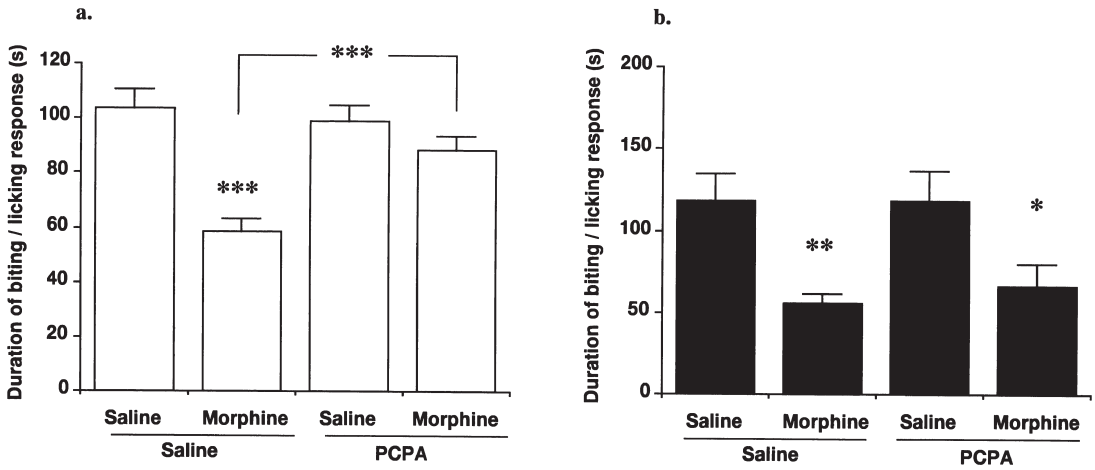

FIG. 2. Effects of pretreatment with *para*-chlorophenylalanine (PCPA) on the first (a) and second (b) phases of morphine analgesia in normal ddY mice. PCPA (800 mg/kg) was administered PO 2 and 3 days before the formalin test, and morphine (10 mg/kg) was administered PO 60 min prior to formalin injection. First phase and second phase represent the summation of nociceptive response time during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM ($n = 10$). Values shown to be statistically significant ($^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$) when compared with controls using a Students' *t*-test.

phase, while not significantly changing the second phase: in the mice treated with PCPA, morphine inhibited the first and second phases by 11 and 44%, respectively, with only the latter change being statistically significant.

Effects of Pindolol, Ketanserin, and Sulpiride on the Antinociceptive Activity of Morphine

In three sets of experiments using normal ddY mice (Figs. 3a, 4a, and 5a), oral administration of morphine (10 mg/kg) induced a comparable and statistically significant inhibition of the first phase of formalin-induced nociception ranging from 34 to 48%. Morphine produced a similar inhibitory effect (36%) on the first phase in mice pretreated with ketanserin (a 5-HT_2 receptor antagonist, 1 mg/kg). This inhibitory effect of morphine on the first phase was, however, significantly attenuated in mice pretreated with pindolol (a 5-HT_1 receptor antagonist, 1 mg/kg) or sulpiride (a DA_2 receptor antagonist, 10 mg/kg), with levels of inhibition reduced to 23 and 18%, respectively.

In the same three sets of experiments using control ddY mice, oral administration of morphine (10 mg/kg) induced comparable and statistically significant inhibition of the second phase of the formalin test, with the inhibition ranging from 49 to 55% (Figs. 3b, 4b and 5b). This inhibitory effect of morphine on the second phase was completely abolished in mice pretreated with sulpiride, but was unchanged in mice pretreated with either pindolol or ketanserin.

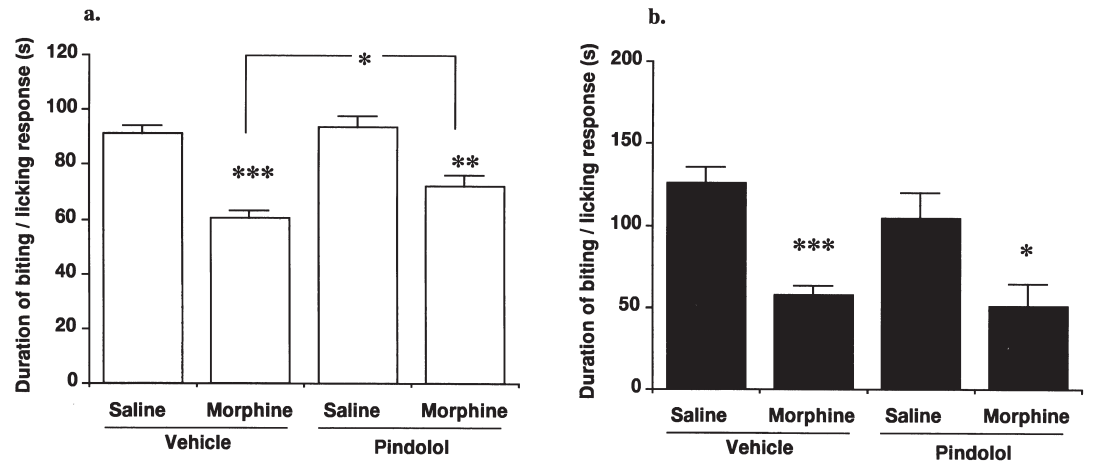

FIG. 3. Effects of pretreatment with pindolol on the first (a) and second (b) phases of morphine analgesia in normal ddY mice. Pindolol (1 mg/kg) was injected IP 30 min before oral administration of morphine (10 mg/kg). First phase and second phase represent the summation of nociceptive response time during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM ($n = 10$). Values shown to be statistically significant ($^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$) when compared with controls using a Students' *t*-test.

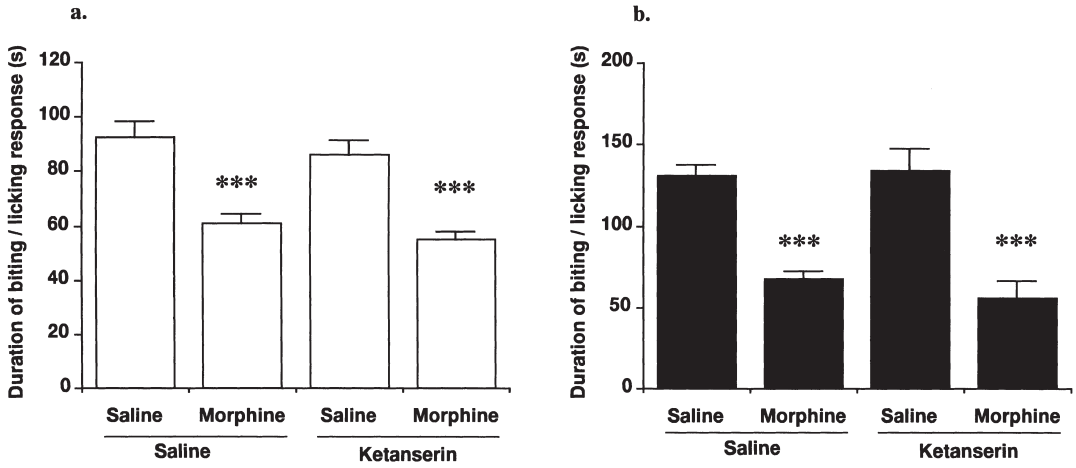

FIG. 4. Effects of pretreatment with ketanserin on the first (a) and second (b) phases of morphine analgesia in normal ddY mice. Ketanserin (1 mg/kg) was injected IP 30 min before oral administration of morphine (10 mg/kg). First phase and second phase represent the summation of nociceptive response time during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM ($n = 9$). Values shown to be statistically significant (***$p < 0.001$) when compared with controls using a Students' *t*-test.

Antinociceptive Effects of Meta-Chlorophenylpiperazine (m-CPP) in Normal and Diabetic Mice

Figure 6 shows the effect of *m*-CPP (a 5-HT receptor agonist) on formalin-induced nociceptive responses in both diabetic and control mice. m-CPP dose dependently reduced the nociceptive response time of both the first and second phases in normal ddY mice (Fig. 6a) being approximately 10 times less potent on the first, $F(4, 40) = 7.16$, $p < 0.001$; $ID_{50} = 3.5$ mg/kg, than the second, $F(4, 40) = 8.48$, $p < 0.001$; $ID_{50} = 0.4$ mg/kg. The drug also caused significant attenuation of the responses in STZ-treated ddY mice [Fig. 6b, first phase; $F(4, 45) = 12.03$, $p < 0.001$, second phase; $F(4, 45) = 4.01$, $p < 0.01$], with ID_{50} values for the first and second phases of 7.8 and 1.0 mg/kg, respectively.

In nondiabetic +/+ mice (Fig. 6c), *m*-CPP significantly reduced the nociceptive response time of the first, $F(4, 35) = 61.05$, $p < 0.001$, and second phases, $F(4, 35) = 64.59$, $p < 0.001$, being slightly potent on the second phase ($ID_{50} = 0.7$ mg/kg) than the first phase ($ID_{50} = 1.3$ mg/kg). Comparable antinociceptive activity of the compound was observed in genetically diabetic db/db mice (Fig. 6d), with ID_{50} values of 0.4 and 1.1 mg/kg for the second, $F(4, 35) = 6.43$, $p < 0.001$, and first, $F(4, 35) = 10.35$, $p < 0.001$ phases, respectively.

Antinociceptive Effects of FR64822 in Normal and Diabetic Mice

Figure 7 shows the effect of FR64822 (a dopaminergic enhancer) on formalin-induced nociceptive responses in both di-

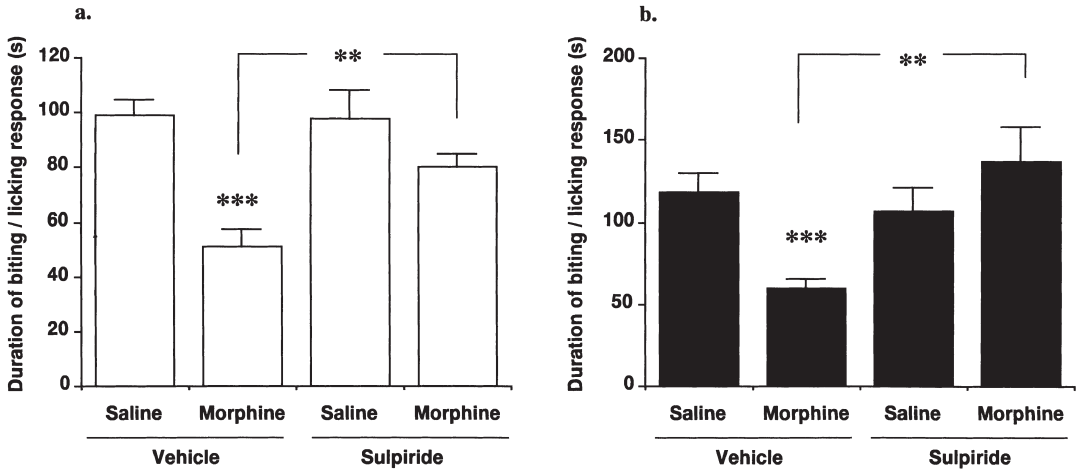

FIG. 5. Effects of pretreatment with sulpiride on the first (a) and second (b) phases of morphine analgesia in normal ddY mice. Sulpiride (10 mg/kg) was injected IP 30 min before oral administration of morphine (10 mg/kg). First phase and second phase represent the summation of nociceptive response time during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM ($n = 10$). Values shown to be statistically significant (**$p < 0.01$ and ***$p < 0.001$) when compared with controls using a Students' *t*-test.

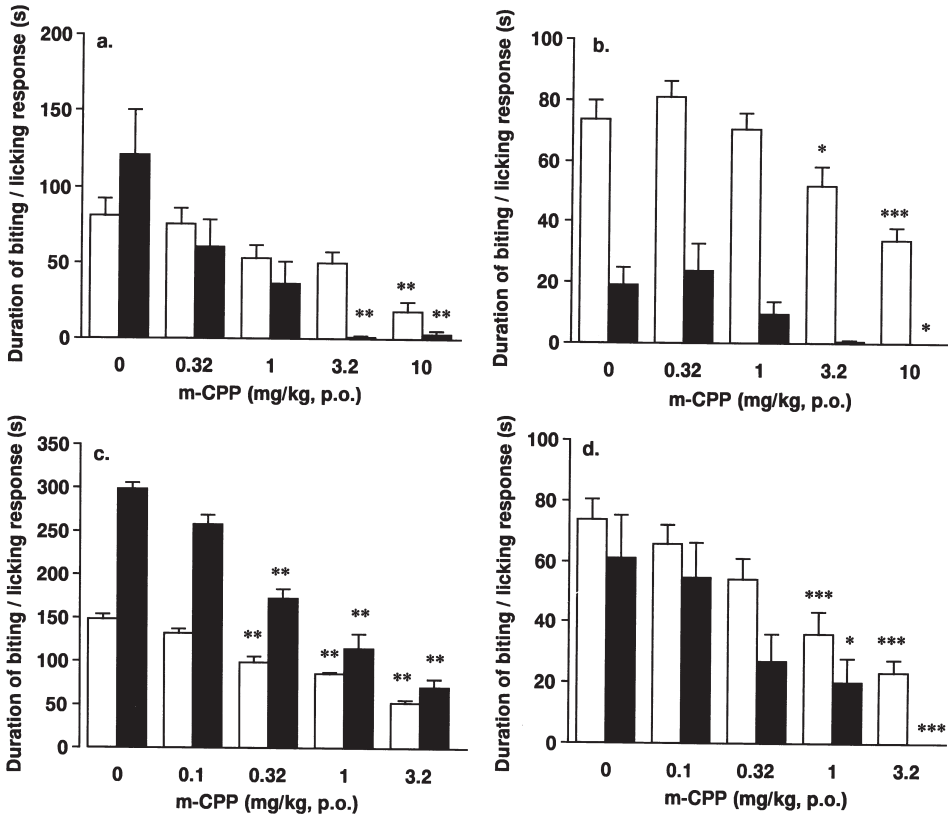

FIG. 6. Effects of *meta*-chlorophenylpiperazine (*m*-CPP) on formalin-induced nociceptive responses in normal ddY (a), STZ-induced diabetic (b), normoglycemic +/+ (c), and genetically diabetic db/db mice (d). *m*-CPP was administered PO 60 min prior to formalin injection. First phase (open columns) and second phase (closed columns) represent the summation of nociceptive responses during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM (n = 8–10). Dunnetts' t-test for multiple comparisons subsequent to ANOVA was used for evaluation. Statistically significant values (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) are shown against their respective 0-mg/kg groups.

abetic and control mice. In normal ddY mice, FR64822 had no significant effect on the nociceptive response time of the first phase up to a dose of 10 mg/kg, $F(3, 36) = 0.45$, $p > 0.05$, but dose dependently inhibited the second phase, $F(3, 36) = 9.47$, $p < 0.001$, with a significant reduction seen at 1 mg/kg (Fig. 7a). The ID_{50} value of FR64822 for the second phase was 2.2 mg/kg. However, in STZ-treated ddY mice, FR64822 had no significant effect on either the first or second phase (Fig. 7b).

In nondiabetic +/+ mice, FR64822 had no significant effect on the first phase, $F(3, 32) = 0.32$, $p > 0.05$, but dose dependently inhibited the second phase, $F(3, 32) = 24.84$, $p < 0.001$, with a significant reduction seen at 1 mg/kg (Fig. 7c). The ID_{50} value of FR64822 for the second phase was 5.5 mg/kg. In genetically diabetic db/db mice, FR64822 had no significant effect on either the first or second phase (Fig. 7d).

DISCUSSION

The present study confirms the original findings by Dubuisson and Dennis (9), who demonstrated that formalin injection into the hindpaw of mice produced a biphasic nociceptive response. In addition, we show that the second phase of this response was significantly attenuated in STZ-treated diabetic mice, and that both phases were significantly attenuated in genetically diabetic db/db mice in comparison with their respective controls. These findings agree with previous reports on STZ-treated mice (16,31) and db/db mice (31), suggesting that pain transmission and/or antinociceptive pathways are differentially affected in the two types of diabetic mice. Given these differences, it is interesting to note that morphine still induces comparable antinociception on the first and the second phases in db/db and normal +/+ mice, considering the previous finding by Greenberg et al. (11) that the levels of methionine-enkephalin immunoreactivity in the pituitaries of db/db mice were significantly higher than in either littermate or background strain controls. Enhancement of the antinociceptive pathways mediated by endogenous opioids could be responsible for the depressed responses in db/db mice. Alternatively, it has recently been suggested that the second phase depends on neural changes that occur during the first phase (3,36,37). It would appear that for STZ-treated mice there may be some alteration in the pain transmission mechanism between the two phases.

Morphine dose dependently attenuated both phases of the formalin-induced nociceptive responses in ddY mice. The antinociceptive effect of morphine on the first phase was significantly reduced by pretreatment with either PCPA (a 5-HT depleter) or sulpiride (a DA_2 receptor antagonist), with the

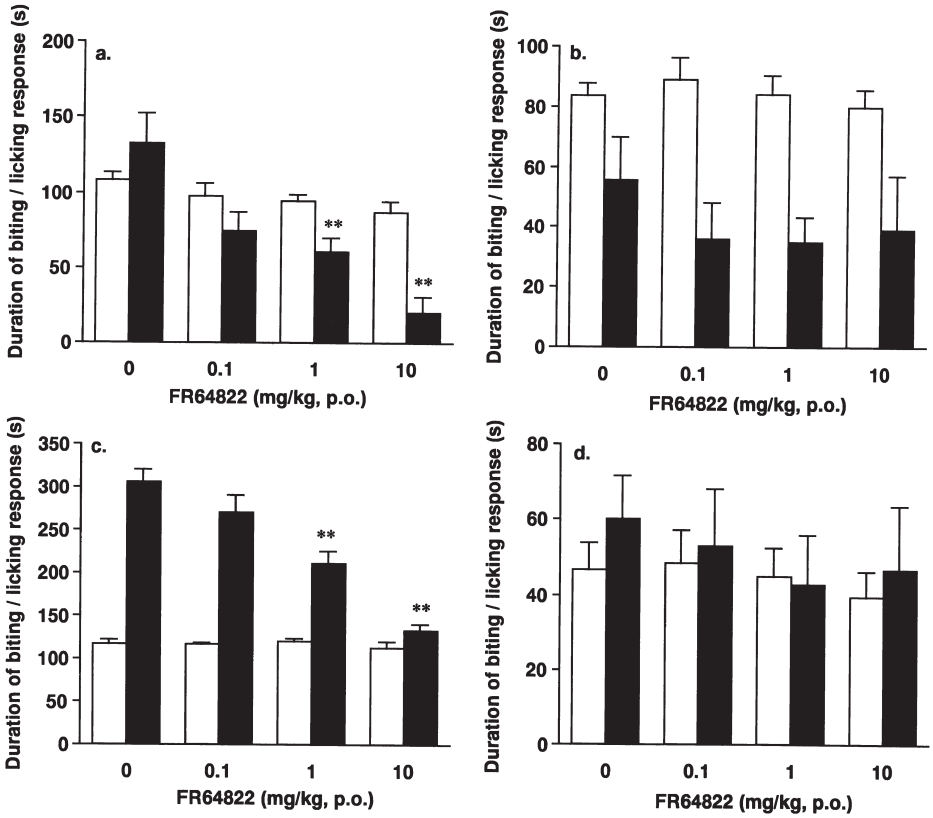

FIG. 7. Effects of FR64822 on formalin-induced nociceptive responses in normal ddY (a), STZ-induced diabetic (b), normoglycemic +/+ (c), and genetically diabetic db/db mice (d). FR64822 was administered PO 60 min prior to formalin injection. First phase (open columns) and second phase (closed columns) represent the summation of nociceptive responses during the 0–10-min and 10–30-min time periods following formalin injection, respectively. Each value represents mean ± SEM (n = 9–10). Dunnetts' t-test for multiple comparisons subsequent to ANOVA was used for evaluation. Statistically significant values ($^{**}p < 0.01$) are shown against their respective 0-mg/kg groups.

latter compound also significantly reducing the effect of morphine on the second phase. These results suggest that dopaminergic and serotonergic mediation are potentially involved in the effect of morphine on the first phase, while dopaminergic mediation may be involved in the second phase. There is ample evidence indicating that morphine induces its analgesic effects through activation of not only opioid (24) but also DA (21,22), 5-HT (5,6), and NE (7,14) receptors. The effects of alpha adrenergic receptor antagonists such as prazosin and yohimbine on morphine antinociception were not examined in the present study, because the former two drugs can produce antinociception in the formalin test (data not shown). Although a substantial amount of research has been done on the effects of adrenergic agents on morphine analgesia, the results are variable. Several experiments demonstrate antagonism of morphine analgesia by adrenergic systems (25,27), some suggest enhancement (2,25), while others find no significant effect (8,10,12,20). Adrenergic mediation of morphine action therefore appears to be complex, and should be clarified by further studies.

It was of interest to find that pindolol (1 mg/kg, a 5-HT_1 receptor antagonist) but not ketanserin (1 mg/kg, a 5-HT_2 receptor antagonist) attenuated the first phase of morphine-induced antinociception in the formalin test in ddY mice. We have shown previously that these doses of drug significantly reduce the effect of tiapride on formalin-induced nociception (31). Several lines of evidence indicate that particular subtypes of 5-HT receptor are involved in regulating the forward transmission of nociceptive input at the spinal and supraspinal levels. According to Richardson (26), the 5-HT receptor subtype involved in mediating inhibition of secondary nociceptive afferents in the dorsal horn is probably a 5-HT_1-like receptor, while a 5-HT_2 subtype no doubt plays a major role in the brain stem. Morphine may, therefore, selectively stimulate the former mechanism to inhibit formalin-induced nociception.

Extending previous findings using the tail-flick and tail-pinch tests (15,30), the present study demonstrates to our knowledge for the first time that the antinociceptive activity of morphine determined using the formalin test was attenuated in STZ-treated mice in comparison with control mice. There is speculation that hyperglycemia induced by diabetes might interfere with the action of morphine on the opioid receptor (30). In support of this, Brase et al. showed that glucose caused small but significant concentration-dependent decreases in opiate receptor affinities for [^{3}H]naloxone and [^{3}H]dihydromorphine binding (1). However, the affinity did not differ between STZ-treated and control mice, suggesting that factors downstream of opioid receptors may be affected

in diabetes. FR64822, a dopaminergic enhancer (23), exerted little antinociceptive activity in STZ-induced diabetic mice using the formalin test, exemplifying the complexity of the situation. It may, therefore, be possible to speculate that a dysfunction of the dopaminergic nerves could be involved, at least in part, for the hyporesponsiveness of STZ-treated mice to morphine. In support of this hypothesis, dopamine turnover was shown to be depressed in STZ-induced diabetic mice (29,33). However, the involvement of reduced 5-HT turnover reported in STZ-treated rats (4,18,35) is ruled out because *m*-CPP and tiapride caused similarly potent antinociception in STZ-treated and control mice when using the formalin test (31).

It is a novel and interesting finding that genetically diabetic db/db mice and their nondiabetic littermates (+/+) are equally responsive to the antinociceptive effect of morphine. In contrast, Simon and Dewey demonstrated that the effect of morphine was reduced in db/db mice when compared with controls (30); however, different methodology may be a contributing factor, as we used the formalin test and they used the tail-flick test. The formalin test is a model of injury-produced pain that has pharmacological characteristics that differ from the reflex withdrawal pain tests, such as the tail-flick test (13,19,21). Whatever the true cause of the contradiction is, our present findings indicate that morphine-induced antinociceptive activity is reduced in STZ-treated mice, but not in db/db mice, when compared with respective controls. This is despite the fact that both types of diabetic mice have hyperglycemia. In this respect, it may be of interest to note that db/db mice have hyperinsulinemia (17). We have recently found that insulin attenuated the formalin-induced nociception in ddY mice in a manner antagonizable with naloxone (an opioid receptor antagonist), sulpiride (a DA_2 receptor antagonist), pindolol (a 5-HT_1 receptor antagonist) and ketanserin (a 5-HT_2 receptor antagonist) (32). Whereas in db/db mice the use of FR64822, m-CPP and morphine indicated that the antinociceptive mechanisms mediated by DA, 5-HT, and endogenous opioids appear to be depressed, unchanged, and enhanced, respectively. It is, therefore, possible to speculate that morphine causes strong antinociception in db/db mice through a mechanism affected by hyperinsulinemia, there by activating 5-HT- and opioid-mediated antinociceptive mechanisms to compensate for the dopaminergic alterations that may be due to hyperglycemia.

In conclusion, morphine attenuated the formalin-induced nociceptive response in a manner antagonizable with PCPA, pindolol, and sulpiride. The effect of morphine was reduced in STZ-induced diabetic mice, but not in genetically diabetic db/db mice. In addition, the effect of FR64822, but not *m*-CPP, on the nociceptive response was reduced in both STZ-diabetic and genetically diabetic db/db mice. Therefore, we speculate that an antinociceptive pathway potentially mediated through dopamine is altered in both types of diabetic mice, and that the attenuated effects of morphine might be due to a dopaminergic dysfunction in STZ mice, and that there might be other mechanisms compensating for the attenuation of dopaminergic function in db/db mice.

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