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BRIEF COMMUNICATION

Effects of Methadone on Activity and on Brain Monoamines in Two Strains of Mice[1]

LAWRENCE D. MIDDAUGH AND JOHN W. ZEMP

Departments of Biochemistry and Psychiatry and Behavioral Science
Medical University of South Carolina, Charleston, SC 29401 USA

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MIDDAUGH, L. D. AND J. W. ZEMP. *Effects of methadone on activity and on brain monoamines in two strains of mice.* PHARMAC. BIOCHEM. BEHAV. 5(3) 367–370, 1976. – Two strains of mice (C57BL/6J and DBA/2J) were used to determine the effects of single and multiple injections of methadone on open field activity and on brain monoamines. For the DBA strain, the initial injection of methadone produced an attenuation of locomotor activity. After 7 daily injections, activity increased to that of controls. For the C57 strain, the initial injection produced a slight increase in activity which became more pronounced after further daily injections. Norepinephrine concentration was elevated in brains of DBA mice chronically exposed to methadone. This effect was not observed in C57 mice nor in either strain injected only once with the drug. Serotonin concentration was not altered in animals of either strain whether acutely or chronically exposed to methadone. The results of this study suggest: 1) that activity change following methadone injections is dependent upon genetic factors and previous experience with the drug; 2) that the tolerance develops to the drug produced decreases but not increases in activity; and 3) that chronic exposure to the drug can elevate norepinephrine concentration in brains of DBA mice.

Methadone Narcotic analgesics Brain monoamines Open field activity

BEHAVIORALLY, methadone has been reported to increase [13,17] or decrease [13] locomotor activity in mice after a single injection depending on the strain of animal used. Evidence of tolerance to some of the behavioral effects of methadone has also been reported. Tolerance to the analgesic effects of methadone has been noted following two weeks of daily injections in rats [6] and 29 daily injections in mice [11]. Swiss-Webster mice made tolerant to levorphanol [17] or injected with methadone for two weeks prior to testing [13] did not show the extensive elevations in activity noted in animals following initial injections of the drug.

Biochemically, acute exposure to methadone has been reported to lower catecholamine levels in the brains of Swiss-Webster mice [17], increase synthesis and degradation of dopamine in rats [1, 2, 16, 18] and increase levels of the serotonin metabolite, 5-hydroxy-indoleacetic acid, in albino mice of unspecified strain [5]. The lowered catecholamine levels reported for mice initially exposed to methadone was absent when the drug was administered to mice previously made tolerant to levorphanol. Levels of 5-HIAA however were further increased following chronic exposure to methadone.

In the study reported here, the effects of acute and chronic exposure to methadone on open field activity and on norepinephrine and serotonin concentration in brains of C57BL/6J (C57) and DBA/2J (DBA) mice were examined. C57 and DBA mice have previously been reported to have elevated and decreased locomotor activity respectively following initial exposure to methadone at 15 mg/kg body weight [13] or to morphine at several doses [8,15].

METHOD

Animals and Treatments

Male mice of the C57 and DBA strains approximately 90 days old at the beginning of the experiment were used. Animals were maintained 4 per cage in a temperature regulated (23° ± 2°C) room on a 12 hr light:12 hr dark cycle with lights on at 0700 hr for at least 21 days prior to the study. Animals were randomly assigned to 1 of 3 treatment groups with 8 animals per group. Drug animals (D) received daily subcutaneous injections of the methadone hydrochloride (Dolophine) (15 mg/kg) dissolved in normal saline. Saline control animals (SC) received daily injections in volumes equivalent to that of the drug group,

[1] Based in part on a paper presented with C. A. Santos, III at the 57th Annual FASEB Meeting, 1973.

0.0075 ml/g body weight. A third group served as uninjected controls (UC).

Behavioral Measures and Procedure

Locomotor activity in an open field arena was assessed by placing the animal in a 40.64 cm × 40.64 cm box with the floor marked off in 10.16 cm squares. The procedure on test days included giving the animal its appropriate injection, isolating it for 5 min, then placing it in one corner of the arena and recording the number of squares entered over a 3 min interval. Five activity tests were made between 0900 hr and 1200 hr after the initial injection and at weekly intervals for the next 5 weeks. Thus, activity was assessed after the first, eighth, fifteenth, twenty-second, and twenty-ninth injection. On the final test (i.e., after the twenty-ninth injection), animals of the SC group received an initial injection of methadone and their activity was compared to that of the D group to provide a further assessment of acute vs chronic effects of the drug on activity. After the final activity test (~30 min after injection) animals were decapitated and brains were removed and frozen for subsequent monoamine assays.

Biochemical Measures

Brains were assayed for 5-hydroxytryptamine (5-HT) and norepinephrine (NE) content by a modification of the technique of Maickel *et al.* [12]. Each brain sample was homogenized in 5.0 ml of acidified butanol. Samples were shaken for 5 min and centrifuged at 500 × g for 2 min. A 4.0 ml portion of the butanol phase was added to tubes containing 9.0 ml of heptane and 0.5 ml of 0.1N HCl, shaken for 5 min and centrifuged at 500 × g for 2 min. The butanol-heptane phase was then aspirated and discarded, and two separate 0.2 ml aliquots of the HCl phase removed. The first was assayed for 5-HT by adding 1.2 ml of o-phthalaldehyde and boiling for 15 min. The tubes were then cooled to room temperature and the amount of fluorescence was determined using an activation wavelength of 360 nm and an emission wavelength of 470 nm. The amount of 5-HT in the sample was determined by comparison of the fluorescence of the sample with the fluorescence of known amounts of serotonin added to brain tissue which were carried through the extraction procedure. The second aliquot was assayed for NE by the method of Chang [9] and was also corrected for recovery by use of an internal standard.

RESULTS

The results of the first four activity tests are summarized in Fig. 1. These data were analyzed using a 2 (Strains) × 3 (Treatments) × 4 (Time) analysis of variance with repeated measures on the last variable. Of particular interest are significant Treatment differences ($F(2,41) = 50.847$, $p<0.001$), Strain differences ($F(2,41) = 63.176$, $p<0.001$) and a Strain × Treatment interaction ($F(2,41) = 6.070$, $p<0.001$). The Treatment effect is due primarily to heightened activity of C57 mice injected with methadone. No differences in activity occurred between SC and UC animals of either strain for any of the tests. The Strain effect and the Strain × Treatment interaction establish that the strains have different activity levels and respond differently to injections of methadone. Subsequent group comparison analysis via *t* tests established that the Strain effects were due to different activity levels in DBA and C57 mice receiving methadone with no strain difference noted for either SC or UC mice.

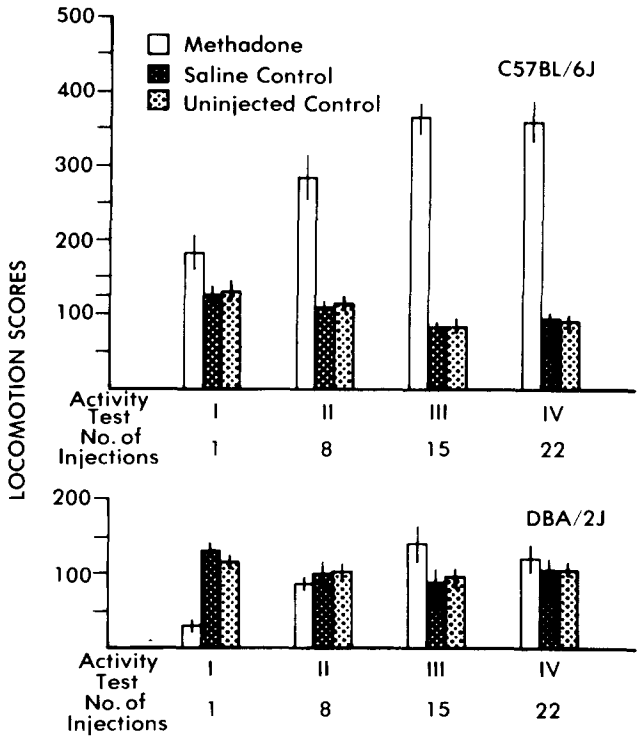

FIG. 1. Mean locomotor scores for C57BL/6J (upper graph) and DBA/2J (lower graph) mice obtained during 3 min activity tests at weekly intervals for 4 weeks. The vertical lines through each bar represent standard error of the mean. Saline control and methadone animals were injected subcutaneously with saline or methadone hydrochloride (15 mg/kg) 5 min prior to each activity test and daily throughout the experiment.

Analysis of the data across time established a significant Time effect ($F(3,123 = 5.398$, $p<0.01$) and Treatment × Time interaction ($F(6,123) = 25.646$, $p<0.001$). Activity levels of UC or SC animals of either strain changed very little demonstrating the reliability of the measuring technique. Animals of both strains injected with methadone, however, showed significant increases in activity between the first and third tests. Although both strains receiving methadone showed activity increases as injections were repeated daily for 3 weeks, the pattern of change was different for the two strains as evidenced by a significant Strain × Treatment × Time interaction ($F(6,123) = 2.384$, $p<0.05$). The major contribution to this effect is a depression in activity following the initial methadone injection in the DBA mice with subsequent return to control levels in contrast to a slight elevation of activity for C57 mice on initial exposure and increasing elevations on subsequent tests.

The results of the final activity and of the monoamine assays are summarized in Table 1. The data were statistically analyzed using 2 (Strain) × 3 (Treatment) analyses of variance and group comparisons via *t* tests with differences at the 0.05 level considered significant.

The results of the activity test are consistent with those reported above with elevated and depressed activity in C57

TABLE 1

EFFECTS OF METHADONE ON ACTIVITY AND ON CONCENTRATION OF MONOAMINES IN BRAINS OF TWO MOUSE STRAINS

	C57BL/6J			DBA/2J		
	Untreated Control $\bar{X} \pm$ SEM	Methadone Acute* $\bar{X} \pm$ SEM	Methadone Chronic† $\bar{X} \pm$ SEM	Untreated Control $\bar{X} \pm$ SEM	Methadone Acute* $\bar{X} \pm$ SEM	Methadone Chronic† $\bar{X} \pm$ SEM
Activity	72 ± 7	193 ± 19	416 ± 23	99 ± 10	27 ± 9	138 ± 30
5-HT¶	902 ± 42	1022 ± 24	856 ± 64	903 ± 60	1034 ± 45	1009 ± 38
NE¶	728 ± 42	724 ± 38	778 ± 28	711 ± 28	689 ± 22	832 ± 16

*Animals received a subcutaneous injection of methadone hydrochloride (15 mg/kg) 5 minutes prior to the activity test.

†Animals were treated as in (a) with the exception of having received 28 daily injections of methadone prior to the activity test.

‡Activity was indexed as the number of squares entered in an open field during a 3 minute test period.

¶Reported as ng/g brain tissue.

and DBA mice respectively, upon initial exposure to methadone. Animals of both strains chronically exposed to the drug also had higher activity levels than those receiving their initial injection.

The results of the monoamine assays also suggest strain differences in reaction to methadone and differences between acute and chronic drug exposure. An analysis of variance established that NE concentration varied according to drug injection and subsequent group comparisons established that chronically exposed mice had significantly higher NE concentrations than either acutely exposed or control mice. Analysis of these data within strains established that DBA mice chronically exposed to methadone had elevated NE concentrations compared to the acute and control groups with no significant differences between treatment groups for C57 mice. No significant group differences in 5-HT concentration were noted for either strain.

DISCUSSION

The main results of this study are that: 1) initial injections of methadone either elevated or depressed activity in the open field depending on the strain tested; 2) mice of either strain had higher activity levels after 7 daily drug injections than after initial drug injection; and 3) NE concentrations were higher in DBA mice receiving multiple drug injections than those receiving a single drug injection.

Elevated and depressed activity in C57 and DBA mice following acute exposure to methadone confirm the results of our earlier report [13] using these strains of mice and a different type of activity measurement. Although the strain difference noted in the current study could depend on dose, similar strain dependent changes in activity have also been reported following acute exposure to morphine at several doses [8,15]. At none of the doses did DBA mice show elevated activity. The activity measure used to assess the effect of morphine on DBA and C57 mice reflected different levels of activity in saline control animals of the two strains, hence the strain difference in drug effect could have been dependent upon initial activity levels. The strain differences noted in the current study on methadone, however, are specifically due to drug injection since no strain differences were noted between either the saline control or uninjected groups. Although depressed activity is commonly observed following injection of narcotic analgesics into rats [1, 2, 14, 16], this effect in mice appears to be restricted to relatively few strains. Aside from the DBA strain, only ddo [10] and CD-1 [14] mice have been reported to have depressed activity following injections of morphine. Other strains so far tested have elevated activity following acute exposure to either methadone [13,17] or morphine [8,15].

Chronic exposure to methadone in the current study produced higher activity levels than acute exposure for mice of both strains. This resulted in convergence toward and divergence from control values for DBA and C57 mice respectively. Convergence toward control values following multiple drug injections is frequently interpreted as representing tolerance [15,17]. Using this criterion, DBA mice in the current study became tolerant to the initial activity depression after repeated injections of the drug. A similar observation was reported for CD-1 mice [14]. In this study initial injections of various doses of morphine sulfate (2.5 mg, 5.0 mg, 10 mg or 20 mg/kg) immediately depressed activity, however, activity tests after the 8th, 15th and 22nd daily injection revealed no drug effect on activity. In the same study, it was reported that after the initial depression, CD-1 mice had elevated activity which showed no evidence of attenuation to repeated drug exposure. In agreement with this study, we observed no attenuation of the excitatory effects of methadone injected into C57 mice. This finding, however, differs from a report that C57 mice become tolerant to the excitatory effects of morphine sulfate upon chronic exposure to the drug [15]. The different effects of chronic drug exposure in the latter two studies might reflect a difference between methadone and morphine, however, is more likely due to differences in drug injection schedules. In the morphine study, C57 mice were injected twice daily whereas in our study and the one on CD-1 mice, they were injected once daily. In another study [19] C57 mice pretreated with morphine sulfate were reported to have greater elevations in activity following subsequent exposure to the drug than those naive to morphine. In this case however, 7 days of no drug injection intervened between pretreatment and subsequent drug test. A further study comparing the effects of the two drugs using the same injection schedules will be necessary

to determine if the sensitization effect noted in the current study on methadone or the tolerance effect noted in the study on morphine are due to drug differences or to injection schedule differences.

The results of the monoamine assays are less marked but do emphasize the different effects of acute and chronic exposure to methadone. In the current study, DBA mice exposed chronically to methadone had higher concentrations of NE than those acutely exposed to the drug. This effect was confined to the DBA strain with no significant group differences in C57 mice. In another study [17], Swiss-Webster mice injected with methadone were reported to have reduced brain catecholamine levels, an effect that was absent in mice pretreated with levorphanol. This result is not necessarily incompatible with our finding since in our study no measures of dopamine (DA) were taken and in the other study using Swiss-Webster mice NE and DA were not isolated from one another. It is possible that DA could be reduced to the extent that it would mask slight increases in NE. Further, methadone at the dose used in our study produced activity changes in DBA mice quite different from that reported for Swiss-Webster mice, hence, a strain difference in the effect of methadone on catecholamine systems might be anticipated.

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