Pharmacology Biochemistry & Behavior, Vol. 32, pp. 241–247. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

The Role of Control Activity Levels in the Reported Strain Differences to the Behavioral Effects of Drugs in Mice[1,2]

GALEN R. WENGER

Department of Pharmacology and Interdisciplinary Toxicology, Mail Slot 611
University of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205

Received 1 February 1988

WENGER, G. R. *The role of control activity levels in the reported strain differences to the behavioral effects of drugs in mice.* PHARMACOL BIOCHEM BEHAV 32(1) 241–247, 1989.—The effect of *d*-amphetamine (3–100 μmoles/kg), scopolamine (0.3–100 μmoles/kg) and morphine (3–1000 μmoles/kg) were studied on the spontaneous motor activity (SMA) of four strains of mice: CF-1, DBA/2, C57BL/6 and CD-1. All three drugs increased the SMA of the CF-1, C57BL/6, and CD-1 strains at low to moderate doses and decreased SMA at higher doses. In the DBA/2 strain, *d*-amphetamine and scopolamine increased SMA at low doses and decreased SMA at high doses; only decreases in SMA were observed with morphine. When the drug effect was expressed relative to control levels of SMA, large apparent strain differences were shown to exist for all three drugs. In general, these strain differences were shown to exist for all three drugs. However, the majority of these strain differences could be attributed to the large differences which existed in the control level of SMA among the four strains. One important exception to this statement was shown to exist. The DBA strain responded differently (only decreases in SMA were observed) to morphine than did the other three strains. This decrease was not related to the control SMA level and could not be antagonized by naloxone (3 μmoles/kg, IP).

Mouse strains C57BL CD-1 CF-1 DBA Morphine Scopolamine *d*-Amphetamine
Motor activity Rate-dependency

THE availability of pure genetic strains of mice has resulted in an abundance of data on the response of the various strains to centrally-acting drugs. Many studies have reported both quantitative and qualitative differences in the behavioral response across strains to many drugs. For example, Kuchinsky (9) reported a 15-fold increase in the spontaneous motor activity (SMA) of Swiss mice following a dose of 70 μmoles/kg morphine (approximately 27 mg/kg morphine sulfate). At the same dose, Castellano *et al.* (2) reported the SMA of DBA mice to be unchanged, but that of C57BL mice to be increased approximately 4-fold. In a similar study (3), a dose of 53.2 μmoles/kg morphine produced a 4-fold increase in SMA in C57BL mice, a 2-fold increase in BALB/c mice, and a slight decrease in DBA mice. Another study (6) on SMA reported large increases in C57BL mice during the first hour following morphine administration. However, in DBA mice only decreases in activity were seen during the same time period following morphine administration. Similarly, differences have been reported for *d*-amphetamine. At a dose of 54 μmoles/kg *d*-amphetamine (approximately 10 mg/kg *d*-amphetamine sulfate), a 27-fold increase in SMA of Swiss mice was reported (12), a 6-fold increase was reported in CF-1 mice (7), and in C57BL mice (1) this same dose produced only a 1.2-fold increase in SMA.

Upon close examination of these studies, it becomes apparent that at least in some reports large differences exist in the SMA of each strain in the nondrug or control states. For example, in the *d*-amphetamine studies mentioned above, there is an approximately 400-fold difference in the control SMA level reported for the Swiss mice (12) and the C57BL mice (1), and a 70-fold difference in control activity levels is reported between the CF-1 mice (7) and the C57BL mice (1). Similar differences in the control level of activity exist in the morphine studies cited. In another behavioral paradigm, schedule-controlled behavior, the rate or level of the control or nondrug behavior being measured has been shown to be a very important determinant of the behavioral effect measured (8), and differences in the control behavior are important determinants in both the magnitude and direction of the drug effect. To date, there have been only a few attempts to determine if rate-dependent effects influence the effects of drugs on SMA. Such a finding would provide significant in-

[1]A preliminary abstract of this study appears in Fed. Proc. 39:1096; 1980.
[2]Supported by USPHS Biomedical Research Support Grant No. SO744 05350, and by NIDA Research Grant DA-02251.

sight into reported strain differences in mice especially when different strains are reported to show different control levels of SMA.

The intent of this study was to test the hypothesis that some of the reported strain differences in the effects of drugs on SMA of mice may be due to differences in the control level of SMA. Therefore, four commonly used mouse strains (CD-1, C57BL/6, DBA/2 and CF-1) were studied under identical conditions, thus, making possible a direct comparison of the effect of control SMA on the drug effect measured without the variable of different techniques for measuring SMA, different laboratories, etc. Drugs representing three different pharmacological classes were studied: *d*-amphetamine, scopolamine, morphine. In addition, the antagonism of morphine by naloxone was studied in the four strains. The results of this study show that in many cases the strain differences observed are related to differences in the control activity levels.

METHOD

Subjects

Adult male mice of the CD-1, C57BL/6NCrlBR (C57BL), DBA/2NCrlBR (DBA) and CF-1 strains were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, MA. All mice were maintained on a 12-hour light/dark cycle, and all experimental sessions were conducted during the light phase of the cycle between 08:00 and 17:00 hours. All mice studied in the experiments on SMA were housed 3–4 mice/cage with free access to food and water in their home cages. During the test session the mice had free access to water only.

Apparatus

SMA was measured with an Automex Activity Monitor, Columbus Instruments. The sensitivity setting was adjusted such that a sufficient number of counts were obtained under control conditions, and increases were measured following low doses of *d*-amphetamine (sensitivity setting=7.00). A 25×19 cm polypropylene mouse cage was placed in the center of each monitor, and a single mouse was placed in each cage. The activity of each mouse was monitored for 90 min on Monday, Wednesday and Friday of each week. Any individual mouse was tested at the same time each test day, and mice of each of the four strains were tested in a counter-balanced fashion at four different times during the period from 08:00–17:00 hours.

Drugs

The drugs studied were *d*-amphetamine·SO_4, scopolamine·HBr, morphine·SO_4, and naloxone·HCl. All drug concentrations were made so that the desired dose could be given in an injection volume of 1 ml/100 g of body weight. Drugs were dissolved in 0.9% saline and administered by IP injection. All doses are expressed as μmoles of free base/kg of body weight. The drug was injected immediately before the mice were placed in the activity monitors and the test session started.

d-Amphetamine and scopolamine were never injected more frequently than twice per week; typically Monday and Friday. Morphine was never injected more frequently than once/week. Typically Wednesday's data were used as control. Drug doses for a given drug were administered in a mixed irregular sequence. Following the determination of the dose-response curves for *d*-amphetamine, scopolamine, and morphine, drug-naive mice were used for a redetermination of the morphine dose-response curve in mice receiving either 3 μmoles/kg naloxone or mice receiving an equal volume of saline.

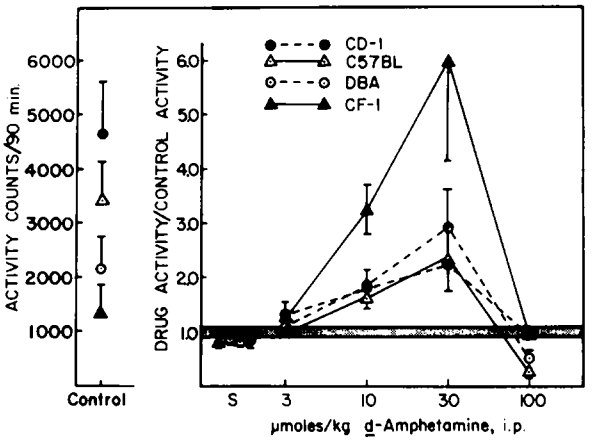

FIG. 1. The effects of *d*-amphetamine on the SMA of mice of the CD-1, C57BL, DBA and CF-1 strains. Abscissa: dose of *d*-amphetamine in μmoles/kg of body weight on a log scale; ordinate: left-side—total activity counts per 90 min test session, right-side—drug effect plotted as a ratio of the activity counts after drug administration divided by the activity counts under control conditions. Points and brackets above control represent the mean±S.E.M. for each strain. Points above S represent the mean±S.E.M. following saline administration. The shaded area around a drug activity/control activity of 1.0 represent ± the largest S.E.M. observed in any of the four strains. The S.E.M. for the other three strains falls within these limits. Points and brackets for all drug points represent the mean±S.E.M. The points for the CD-1 strain represent the mean of 5 mice, the points for the C57BL and DBA strains represent the mean of 6 mice, and the points for the CF-1 strain represent the mean of 5 mice. Ten μmoles/kg *d*-amphetamine=1.85 mg/kg *d*-amphetamine·SO_4.

Measurement of Drug Effects

Control levels of SMA are expressed in terms of total number of activity counts/90-min test session. The effects of saline and drug administration are expressed as a ratio of the total number of activity counts/90 min following drug or saline administration divided by the mean number of total activity counts/90-min control session. For the analysis of the dependency of the drug effect on the control activity level, the total activity counts/90-min session following a given dose of a drug was determined for each mouse. This value was then divided by each mouse's respective mean control value, and the value was plotted for each mouse on a log-log scale as a function of each mouse's own mean control activity counts/90-min session. A regression line was then computed for the data points by the method of least squares.

RESULTS

The effect of *d*-amphetamine on the SMA of each of the four strains is shown in Fig. 1. In addition, the left side of the figure shows the differences which existed in the noninjection control SMA of the four strains. The CF-1 strain was the

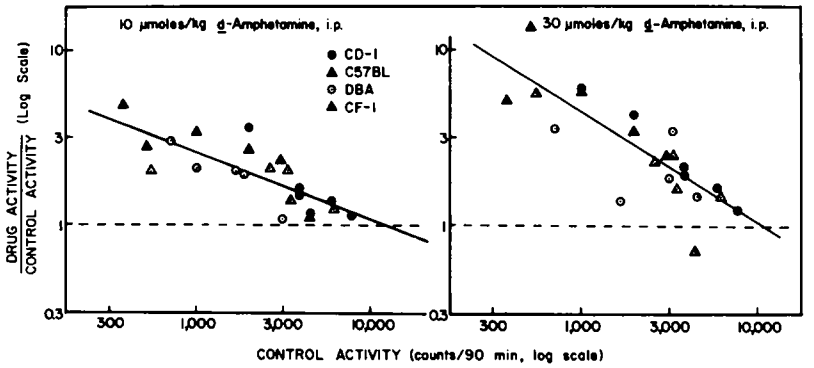

FIG. 2. The dependence of the effect of *d*-amphetamine on the control SMA level in four strains of mice. Abscissa: control SMA counts per 90-min test session on a log scale; ordinate: drug effect plotted as ratio of activity counts after drug administration divided by the activity counts under control conditions on a log scale. Each point represents an individual mouse. A regression line was plotted by the method of least squares.

least active of the four strains, and the CD-1 strain was the most active. The CD-1 strain being approximately 3.5 times as active as the CF-1 strain. The right side of Fig. 1 shows the dose-response curves for *d*-amphetamine in the four strains. When the effect of *d*-amphetamine is plotted for each strain large differences are observed across strains. The largest increases for a given dose are in the CF-1 strain. The CF-1 strain also was the strain which as a group had the lowest control level of activity. Those strains showing higher control levels of SMA, in general, showed smaller relative increases following *d*-amphetamine.

A clearer presentation of this relationship is seen in Fig. 2. Figure 2 shows the effect of 10 and 30 μmoles/kg *d*-amphetamine in each mouse plotted as a function of each mouse's own control level of SMA. When a single least squares regression line is drawn for all the data points at each dose, it becomes apparent that when mice from different strains have approximately equal control SMA values the effect of *d*-amphetamine is approximately equal. For example, after 30 μmoles/kg *d*-amphetamine, the data points at a control activity of 3,000 counts/90 min represent all four strains, and there is very little evidence of a systematic strain difference. The correlation coefficients for the data shown are −0.79 and −0.84 for the 10 and 30 μmoles/kg doses/ respectively.

Figure 3 shows the dose-response curves for scopolamine on the SMA of the four strains of mice. As with *d*-amphetamine, the CF-1 strain having the lowest control activity level shows the largest relative increases in SMA following scopolamine. With the exception of the C57BL strain, the higher the control level of SMA the smaller the increase is for each strain. The mean effect for the C57BL strain is slightly smaller on a relative basis than one might expect based on the mean control rate for the strain.

Figure 4 shows, as in Fig. 2, the effect of two different doses of scopolamine in individual mice of each strain. A single regression line has been drawn for all the data points for all four strains. Following 10 μmoles/kg there is considerably more scatter than seen with *d*-amphetamine. However, with the exception of the points representing the C57BL strain, the points from the other three strains are

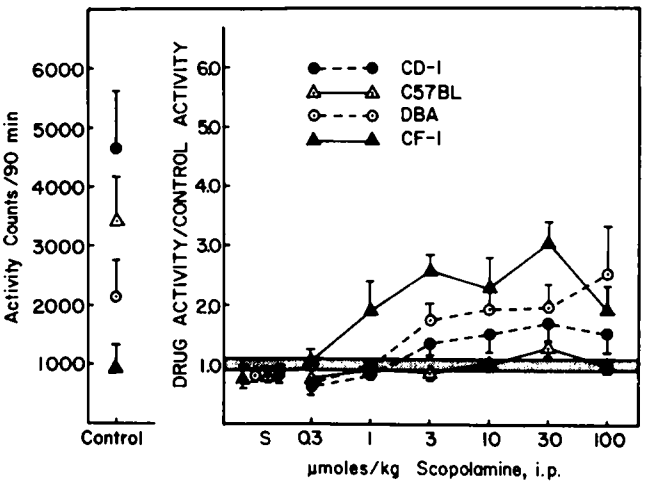

FIG. 3. The effects of scopolamine on the SMA of mice of the CD-1, C57BL, DBA and CF-1 strains. Data plotted as in Fig. 1. The points for the CD-1 strain represent the mean of 5 mice, the points for the C57BL and DBA strains represent the mean of 6 mice, and the points for the CF-1 strain represent the mean of 4 mice. Ten μmoles/kg scopolamine=4.2 mg/kg scopolamine·HBr.

fairly uniformly scattered around the line. The points representing the C57BL strain all lie on the line or below the line. At the next higher dose, however, there is a better fit for the line with a correlation coefficient of −0.79.

The dose-response curves for the effect of morphine in each of the four strains are shown in Fig. 5. Again, the strain with the lowest control SMA level, CF-1, shows the largest relative mean increase in activity following morphine.

With the exception of the DBA strain, the higher the control SMA level the smaller the increase in SMA following morphine. The DBA strain is different. Not only is the SMA not increased as much as would be expected based on the control SMA level, but the mean effect in this strain over a

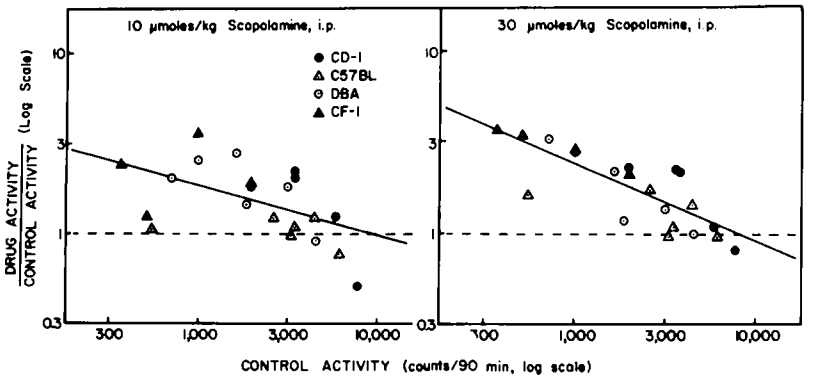

FIG. 4. The dependence of the effect of scopolamine on the control SMA levels in four strains of mice. Abscissa: control SMA counts per 90-min test session on a log scale; ordinate: drug effect plotted as a ratio of activity counts after drug administration divided by activity counts under control conditions on a log scale. Each point represents an individual mouse. A regression line was plotted by the method of least squares.

dose range of 30–300 μmoles/kg is an absolute decrease relative to control. At 1,000 μmoles/kg there is an indication that SMA is returning to control levels.

In Fig. 6 the drug effect in individual mice is plotted as a function of each mouse's respective control activity level. After both 100 and 300 μmoles/kg a single regression line can be drawn for the data points for the CD-1, C57BL and CF-1 strains. In both cases there is a fairly good fit for the line and a correlation coefficient between −0.8 and −0.9. This again suggests that for these three strains the apparent differences in the drug effect across strains is related to the differences in the mean control activity levels of the three strains.

The DBA strain, however, does not appear to fit the same function. For both doses of morphine plotted in Fig. 6, the points representing the DBA mice are clearly separated from the other strains. When a regression line is calculated for the DBA points alone, there is a very low correlation coefficient, and the relationship, if any, which may exist between drug effect and the control activity is very weak.

In an effort to further clarify the differences which may exist between the DBA strain and the other 3 strains in response to morphine, a second series of experiments was started. It was of interest to determine whether naloxone could antagonize the decreases in SMA seen after morphine in the DBA strain to the same extent that it could antagonize the rate increases seen in the CD-1, C57BL and CF-1 strains. Therefore, a naloxone dose-response curve was determined for its effects on SMA in drug naive mice of all four strains. No significant effects were observed in any strain over a dose range of 0.1–100 μmoles/kg naloxone, IP (data not shown).

Following the determination of the naloxone dose-response curves the effect of morphine alone was determined. Figure 7 shows the morphine dose-response curves for SMA (filled circles). In general, the effects seen in Fig. 7 replicate the effects seen in the first group of mice (Fig. 5). The increase in SMA seen in the CF-1 strain is not as large as that observed previously. However, the mean control SMA for the second group of CF-1 mice was higher than observed for the first group (2087±629 vs. 970±366 counts/90 min).

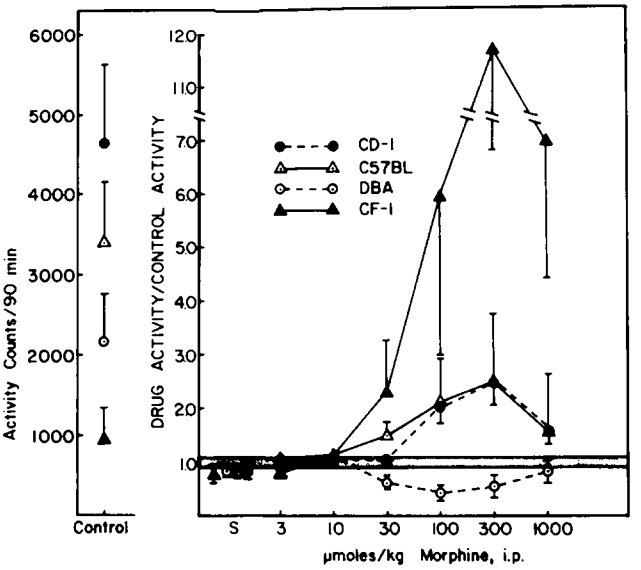

FIG. 5. The effects of morphine on the SMA of mice of the CD-1, C57BL, DBA and CF-1 strains. Data plotted as in Fig. 1. The points for the CD-1 strain represent the mean of 5 mice, the points for the C57BL and DBA strains represent the mean of 6 mice, and the points for the CF-1 strain represent the mean of 4 mice. Ten μmoles/kg morphine = 3.85 mg/kg morphine·SO_4.

The reason for the higher activity level in the second group of CF-1 mice is not clear, but the decrease in the magnitude of the drug effect compared to the first determination supports the hypothesis. In addition, as seen with the first group of DBA mice, morphine decreased SMA relative to control over the dose range of 30–300 μmoles/kg, and the SMA returned to within the control range following 1,000 μmoles/kg.

When 3 μmoles/kg naloxone, IP, was administered immediately prior to morphine, the effects of morphine on

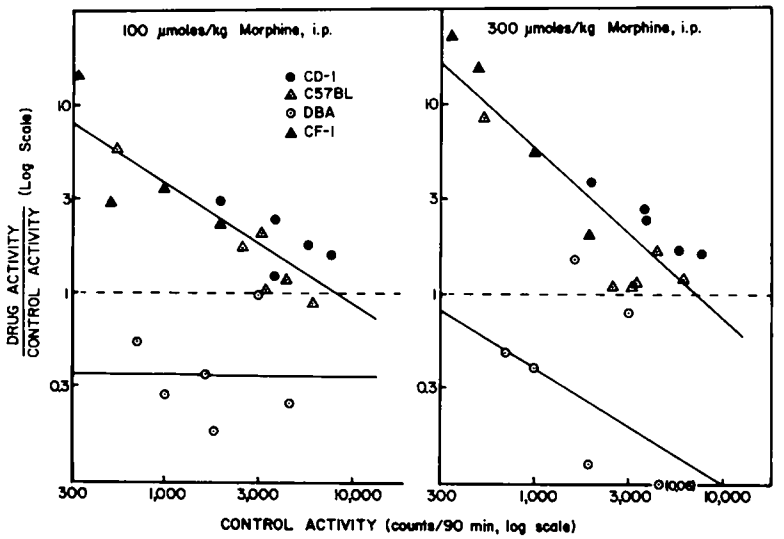

FIG. 6. The dependence of the effect of morphine on the control SMA level in four strains of mice. Abscissa: control SMA counts per 90-min test session on a log scale; ordinate: drug effect plotted as a ratio of activity counts after drug administration divided by activity counts under control conditions on a log scale. Each point represents an individual mouse. A regression line was plotted by the method of least squares.

SMA were antagonized in the CF-1, C57BL and CD-1 strains (open circles, Fig. 7). However, there was little evidence to support a simple antagonism of morphine by naloxone in the DBA strain. When 3 μmoles/kg naloxone was combined with 30 or 100 μmoles/kg morphine, no difference was observed when compared to morphine alone. When the same dose of naloxone was combined with 300 μmoles/kg morphine the decrease in SMA produced by morphine alone was made larger. When combined with 1,000 μmoles/kg, naloxone prevented the return to control SMA levels previously seen in two groups of DBA mice at this dose.

DISCUSSION

The role of the control rate of measured behaviors in the determination of a drug effect on the behavior of an organism has been studied quite extensively (8,10). Over a wide range of drugs of different pharmacological classes, the rate of ongoing control behavior has been shown to be an important variable in the effect of a drug on behavior. Although the role of response rate as a determinant of drug effects was first proposed and studied using the techniques of schedule-controlled behavior, Dews and Wenger (4) reviewed the literature for the effects of amphetamine on a wide variety of behavioral measures in several species and showed the role of control rate in the determination of drug effects to have great generality across behavioral measures.

In a review of the strain difference literature, Wenger (13) suggested that much of the reported differences in the measured drug effects across strains can be accounted for by differences in the behavior under control conditions. That is to say, genetic factors play an important role in determining the amount of behavior which exists under a given experimental condition in the absence of drugs, but once that has been determined differences in the effects of a drug can be attributed largely to the differences in control behavior. Thus, many strain differences could be produced or abolished merely by changing the experimental conditions such that the rate of behavior being measured is either different across strains or equaled across strains. To test this hypothesis directly, four strains have been studied with drugs representing 3 different pharmacological classes. With all three drugs, *d*-amphetamine, scopolamine and morphine, the relative increase in SMA was the largest in the strain having the lowest rate of control SMA (CF-1). In general, the control rate of SMA determined the drug effect in the response of all four strains to 3 different drug classes. This was true for each strain as a group and even more so when the drug effect in each mouse of each of the four strains was plotted as a function of the control SMA in each respective mouse.

One important exception to this general statement must be noted. The response of the DBA strain to morphine was independent of the control rate of SMA. This is in agreement with the study of Gwynn and Domino (6) who reported an increase in SMA in mice of the C57BL strain and a decrease in SMA in DBA mice during the 1st hour after morphine. This observation in DBA mice is important for several reasons. First of all, it demonstrates that the relationship between control SMA and drug effect for the other drugs and strains reported is probably not an artifact of the way in which the data is presented. Secondly, this demonstrates that the control level of activity, although clearly an important determinant of the size and direction of a drug effect, is not the only factor responsible for regulating the subject's response to a drug. Other factors are also important in determining the specific response of a given strain or species to a drug. In the case of the DBA strain it is not totally clear what these other factors are, but this type of analysis has shown that it is not just the difference in the control level of activity which is responsible for the altered response of the

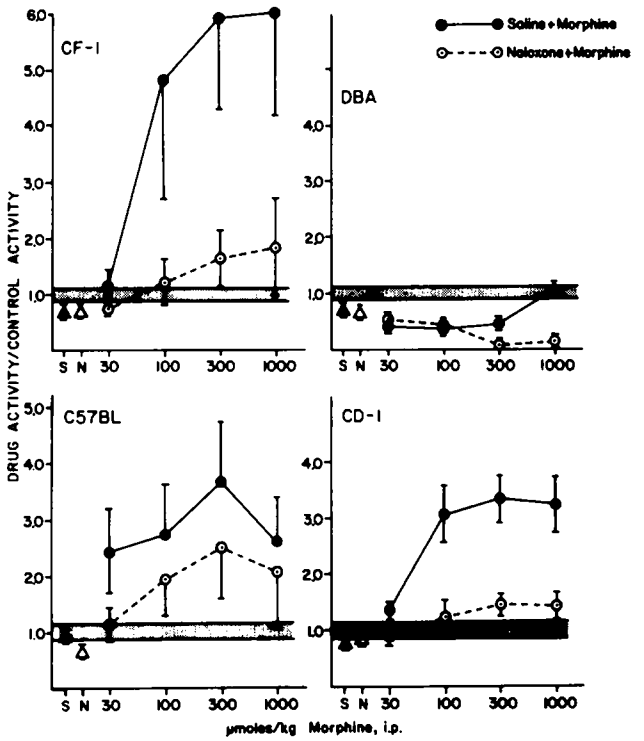

FIG. 7. The antagonism of morphine by naloxone in each of the four strains of mice. Abscissa: dose of morphine in μmoles/kg of body weight on a log scale; ordinate: drug effect plotted as a ratio of the activity counts after drug administration divided by the activity counts under control conditions. The shaded area around a ratio value of 1.0 represents the control mean±S.E.M. Points and brackets above S represent the mean±S.E.M. of duplicate determinations of 2 saline injections given before the test session. Points and brackets above N represent the mean±S.E.M. of the effect on an injection of 3 μmoles/kg naloxone (1.09 mg/kg naloxone·HCl) plus a saline injection. Filled circles and brackets represent the mean± S.E.M. of saline followed by morphine injections. Open circles and brackets represent the mean±S.E.M. of naloxone (3 μmoles/kg) plus morphine injections. n=8 for each strain.

DBA strain to morphine. One clue to the reason for this different response of the DBA strain to morphine may be found in the failure of naloxone to antagonize the effects of morphine on the SMA of the DBA strain. Interestingly, no differences were observed between the four strains in response to naloxone itself.

As noted earlier, differences in the degree of increase in SMA have been reported in different strains of mice having different control levels of activity. However, these previous reports have never examined or discussed this relationship in detail. For example, Eidelberg *et al.* (5) showed that four different mouse strains (C57BL, Swiss, ICR, and BALB/c) mice responded with different degrees of increases in SMA following morphine. Under control conditions, however, there was a nearly 3-fold difference in the levels of SMA with the C57BL strain being the most active, and the BALB/c strain being the least active. If the drug effects reported are plotted as a percent of control, the C57BL strain showed only a very small increase while the BALB/c strain showed the largest percent increase. Thus, this earlier study appears to confirm the hypothesis tested in this study.

The maximum doses used for *d*-amphetamine, scopolamine, and morphine in this study are higher than what one usually sees in similar studies on SMA in mice. Most studies on the effect of morphine on SMA in mice only show increasing SMA with increasing dose, and this author is only aware of one other study that has determined what the maximum SMA response is to morphine in mice (11). In that report, doses up to and including 260 μmoles/kg resulted in increases in SMA of Swiss mice, and the dose response curve only started to turn down at a dose of 780 μmoles/kg morphine. In this study, at doses of 300 μmoles/kg (115.5 mg/kg morphine·SO_4) and lower, there is a relatively monotonic function showing that with increasing dose one gets an increase in SMA. Thus, although the highest doses used in this study appear high compared to the literature, they are necessary to determine the full dose-response relationship. The same argument applies to the doses included in the dose-response curves for scopolamine and *d*-amphetamine.

In conclusion, this study suggests that many of the reported differences in the effects of drugs among strains which are reported in the literature are attributable to a much more general phenomenon: the difference in control behaviors. This could account, as shown in this study, for such observations as the largest relative increases observed for morphine, scopolamine, and *d*-amphetamine being in the strain with the lowest control SMA level, and the degree of response of the other strains being so closely related to a simple ranking of activity levels.

ACKNOWLEDGEMENTS

The author would like to thank Dr. D. E. McMillan for helpful comments during the preparation of this manuscript. I also thank William Hardwick for the preparation of the figures, and Brenda Kay Selby for typing of the manuscript.

REFERENCES

1. Anisman, H.; Wahlsten, D.; Kokkinidis, L. Effects of D-amphetamine and scopolamine before and after shock in three mouse strains. Pharmacol. Biochem. Behav. 3:819–824; 1975.
2. Castellano, C.; Llovera, B. E.; Oliverio, A. Morphine-induced running and analgesia in two strains of mice following septal lesions or modification of brain amines. Naunyn Schmiedebergs Arch. Pharmacol. 288:355–370; 1975.
3. Castellano, C.; Oliverio, A. A genetic analysis of morphine-induced running and analgesia in the mouse. Psychopharmacologia 41:197–200; 1975.
4. Dews, P. B.; Wenger, G. R. Rate-dependency of the behavioral effects of amphetamine. In: Dews, P. B.; Thompson, T., eds. Advances in behavioral pharmacology, vol. 1. New York: Academic Press; 1977:167–227.
5. Eidelberg, E.; Erspamer, R.; Kreinick, C. J.; Harris, J. Genetically determined differences in the effects of morphine on mice. Eur. J. Pharmacol. 32:329–336; 1975.
6. Gwynn, G. J.; Domino, E. F. Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. I. Acute and chronic effects on mouse locomotor activity. J. Pharmacol. Exp. Ther. 231:306–311; 1984.

7. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and *d*-amphetamine. J. Pharmacol. Exp. Ther. 189:51–60; 1974.
8. Kelleher, R. T.; Morse, W. H. Determinants of the specificity of behavioral effects of drugs. Ergeb. Physiol. Biol. Chem. Exp. Pharmacol. 60:1–56; 1968.
9. Kuschinsky, K. Are cholinergic mechanisms involved in morphine effects on motility. Naunyn Schmiedebergs Arch. Pharmacol. 281:167–173; 1974.
10. Morse, W. H.; McKearney, J. W.; Kelleher, R. T. Control of behavior by noxious stimuli. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology, vol. 7. New York: Plenum Press; 1977:151–181.
11. Rethy, C. R.; Smith, C. B.; Villarreal, J. E. Effects of narcotic analgesics upon the locomotor activity and brain catecholamine content of the mouse. J. Pharmacol. Exp. Ther. 176:472–479; 1971.
12. Weissman, A. Effects of amphetamine on locomotion in mice pretreated with desmethylimipramine, tetrabenazine or both. Psychopharmacologia 23:152–159; 1972.
13. Wenger, G. R. Some quantitative behavioral pharmacology in the mouse. In: Thompson, T.; Dews, P. B., eds. Advances in behavioral pharmacology, vol. 2. New York: Academic Press; 1979:1–38.