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Behavioral Effects of Vehicles: DMSO, Ethanol, Tween-20, Tween-80, and Emulphor-620

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CASTRO, C. A., J. B. HOGAN, K. A. BENSON, C. W. SHEHATA AND M. R. LANDAUER. *Behavioral effects of vehicles: DMSO, ethanol, Tween-20, Tween-80, and emulphor-620.* PHARMACOL BIOCHEM BEHAV **50**(4) 521–526, 1995. – Experimental drugs and compounds that do not easily dissolve in water or saline are frequently combined with vehicles like solvents, detergents, or vegetable oils. Yet very little has been reported on the behavioral effects of vehicles. In this study, we assessed the effects of a vegetable oil (emulphor-620), two detergents (Tween-20 and Tween-80), and two solvents [dimethyl sulphoxide (DMSO) and ethanol] on the locomotor activity in CD2F1 male mice. Locomotor activity was monitored for 12 h after vehicle administration (IP). The concentrations for each vehicle were expressed as percent of vehicle in saline (v/v). Emulphor-620 did not affect locomotor activity at any concentration tested (2%, 4%, 8%, 16%, and 32%). Tween-20 significantly decreased locomotor activity at a concentration of 16% and Tween-80 at 32%. DMSO significantly decreased locomotor activity at concentrations of 32% and 64%. In contrast, ethanol produced a biphasic behavioral response: increased activity at a concentration of 16% and decreased activity at a concentration of 32%. These results will facilitate the selection and concentration of vehicles to be used in combination with experimental drugs or test agents.

Locomotor activity Vehicles DMSO Tween-20 Tween-80 Ethanol Emulphor-620

MANY experimental drugs and compounds do not dissolve readily in water or saline and, thus, are difficult to administer. For example, radioprotective eicosanoids such as 16,16 prostaglandin E_2 and misoprostol (12,13,16,17), antitumor agents such as ellipiticine and acronycine (6,28), adenosine derivatives such as N^6-cyclohexyladenosine and 5′-N-ethylcarboxamidoadenosine (8,20), and anticonvulsant drugs such as primidone and carbamazepine (19) can each be administered only in combination with a vehicle. Compounds used most often as vehicles have been solvents, detergents, or vegetable oils (4,10,21). The most commonly used solvent vehicles in both in vivo and in vitro models include dimethyl sulphoxide (DMSO) and ethanol [e.g., (2,26)]. The polyoxyethylene sorbitan monolaurate detergents Tween-20 and Tween-80 are commonly used vehicles in pharmacological and physiological studies [e.g., (5,19,25,27)]. Of the vegetable oil vehicles, the polyethoxylated castor oil emulphor-620 is used most often probably because it is the preferred vehicle for the investigation of pharmacological effects of the much studied cannabinoid compounds [see (18)].

Surprisingly, very little has been reported on how these vehicles affect behavior. This study characterized the behavioral effects of DMSO, ethanol, Tween-20, Tween-80, and emulphor-620 by measuring their effects on the locomotor activity of mice.

METHOD

Subjects

Subjects were male outbred (BALB/c × DBA/2)F1 mice (Charles River Breeding Laboratory, Raleigh, NC), also known as CD2F1 mice, weighing 24–30 g. All mice were quar-

The opinions and assertions contained in this report are the views of the authors and do not necessarily reflect those of the Department of Defense. During the conduct of this study, the investigators adhered to the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication No. 86-23.

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antined on arrival and representative animals were screened for evidence of disease. Mice were housed in groups of 10 in Microisolator cages (Lab Products, Maywood, NJ) on hardwood chip contact bedding in a facility approved by the American Association for Accreditation of Laboratory Animal Care. Animal rooms were maintained at 21 ± 2°C with 50 ± 10% humidity on a 12 L : 12 D cycle. Commercial rodent chow and acidified water (pH 2.5) were freely available.

Apparatus and Procedure

Computerized Digiscan activity monitors (Omnitech Electronics, Columbus, OH) were used to quantify locomotor activity. Each monitor used an array of infrared photodetectors spaced 2.5 cm apart to determine horizontal locomotor activity, which was expressed as the total distance travelled. Immediately after all injections (IP), mice were placed into individual Plexiglas activity chambers (20 × 20 × 30 cm). Activity was continuously monitored every 5 min for the first 2 h to ascertain the onset of effects; thereafter, activity was continuously recorded at 1-h intervals for the next 10 h. All testing took place during the dark portion of the light : dark cycle. Each animal was tested only once. Food and water were available throughout the testing period.

Vehicle Compounds

Saline (0.9% sodium chloride) was obtained from Abbott Laboratories (North Chicago, IL); emulphor-620 (Alkamuls) from Rhone-Poulenc (West Point, GA); ethanol (100%) from Pharmco Products Inc. (Weston, NJ), DMSO; and Tween-20 and Tween-80 from Sigma Chemical Company (St. Louis, MO). Because vehicle concentrations are typically administered as a percent of the diluent, the vehicles used in this study are expressed as percent of vehicle in saline (v/v). We used a 25 gauge needle for each IP injection in a volume of 10 ml/kg.

Data Analysis

Locomotor activity was analyzed using a repeated measures analysis of variance (23). Vehicle compound was the between-subjects effect, and time was the within-subject effect. Separate analyses were made for the minute and hour data and all p-values used were Greenhouse-Geisser corrected (23). When a significant interaction of the main effect and time was present, a Student–Newman–Keuls test (23) was used to assess differences in treatments at each time point. A log transform was used in the analysis to help stabilize variances.

The behavioral median effective dose (ED_{50}) of each vehicle was estimated using weighted least square fits of functional forms of the locomotor activity vs. percent vehicle concentration. The standard error of each ED_{50} was estimated using error propagation, and these errors were then used to compute 95% confidence intervals. The ED_{50} was defined as the percent concentration of the vehicle in saline (v/v) that reduced locomotor activity to 50% of saline control value. ED_{50} values were calculated at 1 and 2 h after vehicle administration.

RESULTS

Behavioral ED_{50}s

The behavioral ED_{50}s, expressed as a percentage of vehicle in saline, and the confidence intervals of each vehicle at 1 and 2 h after injection are presented in Table 1. Precise ED_{50}s could not be determined for emulphor-620 at either 1 or 2 h after injection because at the highest concentration tested (32%) it did not reduce locomotor activity by at least 50%. For the same reason, an exact ED_{50} could not be determined for Tween-80 at 1 h after injection. Higher concentrations of these two vehicles were not evaluated because their viscous properties prevented IP administration with a 25 gauge needle.

Saline

The locomotor activity of mice administered saline is compared with that of noninjected mice in Figs. 1A and 2A. The total distance travelled by each of these groups of mice was not significantly different, (all p-values > 0.61), indicating that the injection procedures had no effect on locomotor activity. However, both the minute and hour data indicated that activity in both groups decreased over time, all F-values > 22.14, all p-values < 0.0001.

Vegetable Oil (Emulphor-620)

Emulphor-620 did not alter the distance travelled by the mice at any concentration tested. For both the minute and hour data (Figs. 1B and 2B), neither the main effect of emulphor-620 nor the emulphor-620 × time interactions were significant, all p-values > 0.18. The main effect of time, how-

TABLE 1

EFFECTIVE DOSES (ED_{50}) AND CONFIDENCE INTERVALS (CI) FOR LOCOMOTOR BEHAVIOR IN MICE EXPRESSED AS PERCENTAGE OF VEHICLE IN SALINE

		Behavioral ED_{50}s and CIs*	
Vehicle	Chemical Name	% at 1 h	% at 2 h
Emulphor	Polyethoxylated castor oil	> 32.0	> 32.0
Tween-20	Polyoxyethylene sorbitan monolaurate	9.6 (8.4–11.0)	14.4 (11.1–18.7)
Tween-80	Polyoxyethylene sorbitan monooleate	> 32.0	30.1 (28.9–31.3)
DMSO	Dimethyl sulfoxide or sulfinylbis [methane]	27.0 (24.4–29.9)	32.3 (30.3–34.4)
Ethanol	Ethyl alcohol	27.7 (26.6–28.3)	31.25 (30.2–32.3)

*ED_{50}s and CIs were calculated at 1 and 2 h after IP administration.

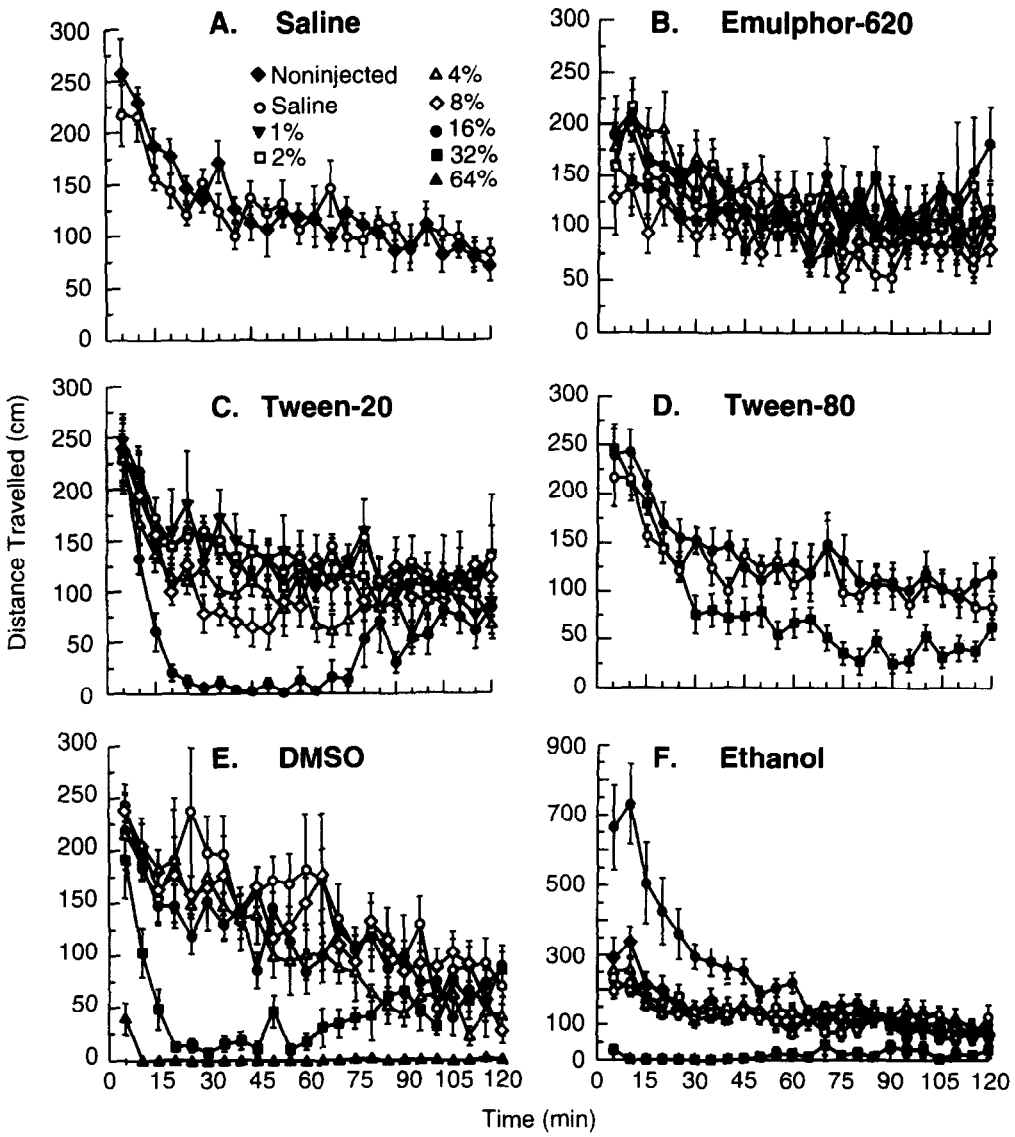

FIG. 1. Onset of locomotor behavior expressed as distance travelled (mean ± SEM) 5–120 min after administration (IP) of six commonly used drug vehicles. All vehicle compounds were administered as percent of vehicle in saline (v/v). n = 8–12 mice per group.

ever, was significant, all F- values > 26.24, all p-values < 0.0001, indicating again that the activity of the mice in both groups decreased during the testing period.

Detergents (Tween-20 and Tween-80)

Only the highest Tween-20 concentration tested (16%) significantly decreased the distance travelled by the mice. As illustrated in Figs. 1C and 2C the Tween-20 × time interactions were significant for both the minute and hour data, all F-values > 1.36, all p-values < 0.05. Post hoc analyses of these interactions indicated that, compared with all other groups tested, the 16% concentration of Tween-20 significantly decreased the distance travelled at 15 to 75 min after administration and, to a lesser extent, decreased the distance travelled for the first 2 h after administration (p-values < 0.05).

Only the 32% concentration of Tween-80 decreased the distance travelled by the mice. As shown in Figs. 1D and 2D, the Tween-80 × time interactions were significant for both the minute and hour data, all F-values > 1.81, all p-values < 0.005. Post hoc analyses revealed that the distance travelled by mice receiving a 32% concentration was significantly decreased at 30 min, 35 min, 50–115 min and 2 h following administration compared with that by mice receiving either a 16% concentration of Tween-80 or saline (p-values < 0.05).

Solvents (DMSO and Ethanol)

The 32% and 64% concentration of DMSO significantly decreased the distance travelled following administration (Figs. 1E and 2E). For both the minute and the hour data, the main of effects of DMSO and time and as well as the DMSO × time interactions were significant, all F-values > 3.27, all p-values < 0.0001. Analyses of the minute data revealed that the distance travelled by the mice receiving a 32% concentra-

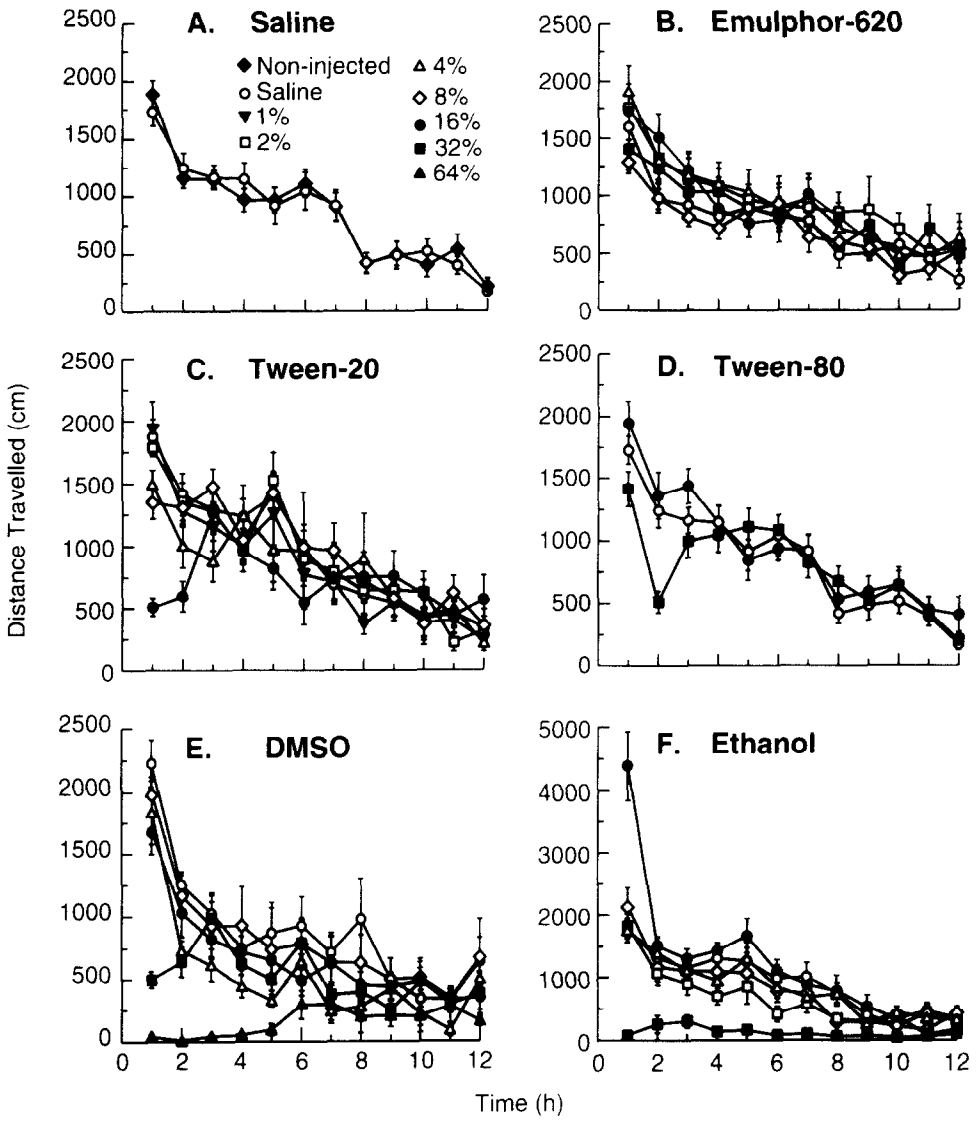

FIG. 2. Duration of locomotor behavior expressed as distance travelled (mean ± SEM) 1-12 h after administration (IP) of six commonly used drug vehicles. All vehicle compounds were administered as percent of vehicle in saline (v/v). n = 8-12 mice per group.

tion was significantly decreased at 10-75 min after administration (p-values < 0.05) and that the distance travelled by the mice receiving a 64% concentration significantly decreased at 5-120 min following administration (p-values < 0.05). Analyses of the hour data indicated that the distance travelled by the mice receiving a 32% concentration was decreased at 1 h and that the distance travelled by the mice receiving a 64% concentration was decreased at 1-6 h (p-values < 0.01).

The effects of ethanol on the distance travelled by the mice were biphasic (Figs. 1F and 2F). The activity increased after administration of a 16% concentration and decreased after administration of a 32% concentration. Consistent with this description of the data, the main effects of ethanol and time as well as the ethanol × time interactions were significant, all F-values > 1.70, all p-values < 0.002. Post hoc analyses of the ethanol × time interactions for the minute data revealed that the mice receiving a 16% concentration travelled a greater distance than all other groups at 5-45 min after administration (p-values < 0.05). Analyses also revealed that the mice receiving a 32% concentration travelled a lesser distance than all other groups at 5-120 min after administration (p-values < 0.05). Post hoc analyses of the ethanol × time interactions for the hour data indicated that the mice receiving a 16% concentration travelled a greater distance than all other groups only at 1 h after administration (p-values < 0.01) and that the mice receiving a 32% concentration travelled a lesser distance than all other groups at 1-8 h after administration (p-values < 0.01).

DISCUSSION

Three major classes of compounds used as vehicles for drugs are vegetable oils, detergents, and solvents. This study assessed the effects of representative compounds from each of these classes on the locomotor activity in mice. Our results demonstrate that the vegetable oil emulphor-620 did not affect

the locomotor activity at any concentration tested (2–32%), that the detergents Tween-20 and Tween-80 depressed locomotor activity at respective concentrations of 16% and 32%, that the solvent DMSO decreased locomotor activity at concentrations of 32% and 64%, and that the solvent ethanol had a biphasic effect, increasing locomotor activity at a concentration of 16% and decreasing it at a concentration of 32%. Because emulphor-620 did not affect locomotor activity in our study, it may be better than the solvents or detergents tested in this study when behavioral toxicity is considered as an end point. Ashby and Mirkora (2) have recommended that vegetable oils replace DMSO as a vehicle for insoluble compounds. Kocsis et al. (15) have shown that DMSO reduces locomotor activity, and Fossom et al. (9) demonstrated that it impairs operant performance in the rat.

Vegetable oils, however, may not always be the vehicles of choice. Emulphor-620 has been reported to alter serum lipid levels, lipoprotein patterns, and tissue lipid content in dogs (11). Indeed, in many instances a detergent or solvent vehicle is preferred. For example, Tween-80 was found to be the only adequate solubilizer for use in smooth muscle preparations when compared with ten other vehicles including DMSO and ethanol (4). Moreover, Tween-80 has been used as a vehicle to evaluate the behavioral effects of experimental drugs and toxicants without apparent adverse side effects (24). Similarly, O'Hara and colleagues (21) found DMSO to be a better vehicle than ethanol or emulphor-620 for evaluating the toxicity of chlorinated hydrocarbons in the isolated hepatocyte system. Therefore, depending on whether the experimental model is in vivo or in vitro and the end point selected, a vehicle compound from any one of the three classes may be appropriate.

Frequently, however, investigators use a combination of vehicles to administer an experimental drug or test agent. Emulphor-620 and ethanol are often used in combination to administer a variety of agents including delta-9-tetrahydrocannabinol (22), fungicides (1), insecticides (3) and antibiotics (28). When these vehicles are used in combination, relatively low concentrations are given, usually consisting of 5% emulphor and 5% ethanol in 90% saline (v/v) (1,3,22,28). Although our results indicated that lower concentrations of emulphor-620 and ethanol alone did not alter locomotor activity (see Figs. 1B, 1F, 2B, 2F), a combination of emulphor-620 and ethanol may. In fact, a vehicle solution consisting of emulphor (5%) and ethanol (5%) has been shown to lower the blood pressure and increase the heart rate of dogs (11). Thus, if more than one vehicle is used to administer an experimental drug or test agent, it is important to determine the effects not only of the individual vehicles but also of the combinations.

A vehicle solution having no apparent effect when administered alone may dramatically alter the response of the drug being investigated. For example, DMSO reportedly precipitates crystallization or deposition of experimental drugs and test agents upon contact with biological fluids, thus extending its time course (2). DMSO has also been shown to lower the sensitivity of rats to the rate-decreasing effects of *d*-amphetamine on an operant behavioral task (9) and to enhance the toxicity of the antineoplastic agent bleomycin (14). Similarly, Tween-80 has been shown to lower the anticonvulsant effects of primidone and carbamazepine because it reduces drug absorption (19), and Tween-20 has been shown to decrease the bioavailabilty of a benzodiazepine receptor ligand 20-fold compared with the vehicle polyethylene glycol (PEG-400) (18). Thus, because vehicle solutions can alter the bioavailability and increase or decrease the toxicity of experimental drugs and compounds, it has been recommended that potency estimations be based not only on the doses administered but also on the brain and plasma concentrations of the experimental drug or test agent (19).

In conclusion, to minimize toxicity, it is best to dissolve experimental drugs and compounds in either saline or water. When this is not possible, it is important to ensure that the vehicle does not affect the response under investigation. Often this will require that the selected vehicle be compared with other vehicle compounds, as demonstrated in this study.

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