Pharmacology Biochemistry & Behavior, Vol. 18, pp. 821–824, 1983. © Ankho International. Printed in the U.S.A.

BRIEF COMMUNICATION

Parachloroamphetamine Toxicity in Mice: Influence of Body Weight, Sex and Dose[1]

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Received 28 October 1982

KESSELRING, J., R. G. SEWELL, J. A. GALLUS, T. R. STIGER AND N. I. NEARCHOU. *Parachloroamphetamine toxicity in mice: Influence of body weight, sex and dose.* PHARMACOL BIOCHEM BEHAV **18**(5) 821–824, 1983.—Five doses of *d,l*-para-chloroamphetamine (0.0, 15.0, 30.0, 45.0 and 60.0 mg/kg) were used to challenge 10 groups of 16 male and 10 groups of 16 female CF-1 mice weighing either 16 to 25 g or 40 to 55 g. Twenty-four hours after intraperitoneal injection, rates of lethal toxicity were assessed. Effects for body weight and dose were found. In addition, a sex by dose interaction was demonstrated. It was hypothesized that the influence of weight might be related to thermoregulatory processes, since as weight rises, surface-to-volume ratios decline, and with them the efficiency of heat exchange. Caution is suggested in the interpretation of ontological studies of drug response.

Parachloroamphetamine Mice Drug toxicity Body weight Sex Lethality Subject variables

DRUG toxicity has been increasingly demonstrated to be a function of widely ranging variables, in addition to parameters of the agent in question. Nutritional (e.g. [2,4]), pharmacological (e.g. [11,16]), physiological (e.g. [20,26]), and environmental (e.g. [18,26]) modulators have been explored. Taken collectively, such data emphasize that a drug's lethal dose characteristics e.g., (LD_{50} the dose of drug at which 50% of subjects are likely to die) should not be considered as immutable properties of the drug. Rather, the LD_{50} index appears to be highly specific to the organism's physiological state and environmental context.

In a recent study of *d,l*-parachloroamphetamine (PCA) toxicity with rats, both social and non-social environmental variables were found to exert strong modulatory influences [7]. Subsequently we attempted to extend these findings to a second species, mice, as Neilson, *et al.* [17] have shown PCA to yield toxic effects in this animal. In so doing, we adventitiously noted that which appeared to be an influence of body weight upon PCA-induced lethality. Various previous reports have demonstrated weight as influential in toxicity studies employing other agents (e.g., *d*-amphetamine 2,3; acivicin, 15). We therefore explored directly the relationship between body weight and PCA-induced lethality, employing both male and female CF-1 mice.

METHOD

Subjects

One hundred sixty male and 160 female CF-1 mice served as subjects. For each sex, two weight ranges were included, with one-half the subjects weighing 16–25 g and one-half weighing 40–55 g. The mice were born in this laboratory's colony and raised in groups of 10 to 25 subjects per cage, with continuous access to food and water. The line of mice maintained in our colony was initially derived from The Upjohn Co. (Kalamazoo, MI) CF-1 stock. Illumination within the colony room was constant and the temperature was maintained at 25–28°C.

Apparatus, Procedure and Drug Preparation

The influence of sex, body weight and dosage level upon the lethality of *d,l*-para-chloroamphetamine (PCA) in group-housed mice was examined. At each of five PCA dosage levels (0.0, 15.0, 30.0, 45.0 and 60.0 mg/kg) four groups of mice, each with different subject characteristics, were tested. These four groups consisted of two male groups and two female groups comprised of either "light" (16 to 25-g)

[1]This research was supported in part by a Graduate Student Research Award to J.W.K. and in part by Department of Psychology assistance to the Laboratory (*). Vivian Farah is here acknowledged for her editorial assistance as is Science Graphics for provision of the figure.

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TABLE 1

DRUG TREATMENT AND SUBJECT CHARACTERISTICS FOR EACH GROUP

Group	Drug	Dose (mg/kg)	Sex	Weight	N
1.	Saline	—	F	Heavy	16
2.	Saline	—	F	Light	16
3.	PCA	15.0	F	Heavy	16
4.	PCA	15.0	F	Light	16
5.	PCA	30.0	F	Heavy	16
6.	PCA	30.0	F	Light	16
7.	PCA	45.0	F	Heavy	16
8.	PCA	45.0	F	Light	16
9.	PCA	60.0	F	Heavy	16
10.	PCA	60.0	F	Light	16
11.	Saline	—	M	Heavy	16
12.	Saline	—	M	Light	16
13.	PCA	15.0	M	Heavy	16
14.	PCA	15.0	M	Light	16
15.	PCA	30.0	M	Heavy	16
16.	PCA	30.0	M	Light	16
17.	PCA	45.0	M	Heavy	16
18.	PCA	45.0	M	Light	16
19.	PCA	60.0	M	Heavy	16
20.	PCA	60.0	M	Light	16

mice or "heavy" (40 to 55-g) mice. Each group contained 16 animals (see Table 1).

PCA administrations were prepared from *d,l*-parachloroamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) and delivered intraperitoneally in isotonic saline solution at volumes of 10 ml/kg. Immediately following injection, the subjects were placed in an aggregate housing condition. The aggregate housing environment consisted of 16 animals from the same subject group, housed in a stainless steel cage measuring 30×22×21 cm (Unifab Corp., Kalamazoo, MI). Purina Laboratory Rodent Chow (Ralston Purina Co., St. Louis, MO) and water remained freely available in the test cage. All testing occurred under conditions of constant temperature and illumination. All drug challenges occurred between the hours of 1:00 and 7:00 p.m. and the number of subject deaths was assessed 24 hours later.

Statistical Methods

The results of this study were statistically analyzed by the method of Grizzle. Starmer and Koch [8]. This technique uses linear models for various functions of categorical data. The computations were performed using the FUNCAT procedure of the S.A.S. computer system. For the analysis, the saline control cells were eliminated. The optimum model, which contained effects of weight, sex, dose, sex-by-dose and sex-by-weight, indicated a significant sex-by-dose interaction. Thus, subsequent analysis was performed for each sex independently by employing a model with the effects of dose and weight.

RESULTS

Presented in Fig. 1 are the results of the experiment. Inspection of the figure reveals an effect of dosage level found

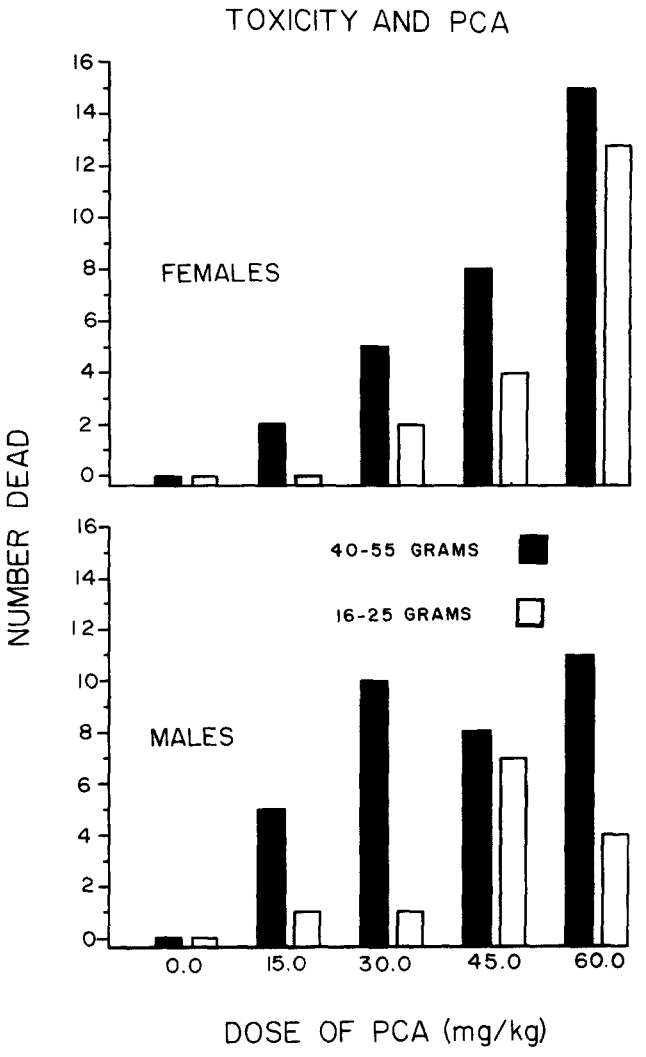

FIG. 1. Number of deaths 24 hours post-injection for groups of 16 mice which were exposed to one of five dosage levels and which possessed the subject characteristics of (1) being either male or female and (2) weighing either 40 to 55 g ("heavy") or 16 to 25 g ("light").

in both sexes and in each of the two weight classes. In general, as dose level increased, so too did the degree of lethal toxicity. In addition, the effect of weight appeared to be unambiguous. At all levels of PCA tested, save saline control, the 40 to 55-g mice experienced a higher mortality rate than that experienced by the 16 to 25-g subjects. This generalization held true for both sexes.

Statistical analysis revealed evidence of main effects for body weight ($p<0.0001$) and PCA dose ($p<0.0001$), but not for sex ($p<0.8573$). However, an interaction of sex by dose was also noted ($p<0.0001$). An interaction was not found for sex by weight ($p<0.1250$). Because the sex by dose interaction did appear, further analysis was conducted for each sex in isolation. For females, weight ($p<0.0188$) and dose ($p<0.0001$) did emerge as significant. These results were also obtained for the male subjects (weight: $p<0.0001$, dose: $p<0.0201$).

DISCUSSION

The present study demonstrated that for both sexes, PCA toxicity was directly related to dosage level. This dose-dependency is in accord with previous PCA toxicity data (e.g. [7,17]. No main effect of sex was found, although a sex-by-dose interaction was discovered. Scrutiny of Fig. 1 reveals that in the dose range of 30 mg/kg and under, the heavy male mice experienced higher death rates than did females of the same weight class. The present finding of differential mortality is consistent with results reported by Södersten *et al.* [23] in which a distinctly higher mortality rate for castrated male as opposed to castrated female rats was seen following treatment with PCA at 10.0 mg/kg.

Unequivocal effects of subject weight were found at each PCA dosage level tested in both sexes. After each PCA challenge, subjects weighing 40 to 55 g sustained higher mortality rates than those weighing 16 to 25 g. Previous studies of *d*-amphetamine have also shown heavy mice to experience higher mortality rates than light mice (e.g. [1,3]). The mechanism by which weight strongly modulated PCA toxicity remains unknown. One potential mediator involves drug-induced hyperthermia. Studies of increased ambient and/or internal body temperature have repeatedly shown hyperthermia to markedly enhance the toxicity of various agents, particularly those with central effects (see, for example, [25]). Previous studies have demonstrated hyperthermia subsequent to PCA treatment [6, 11, 21, 22] (cf. [10]). Related to this is the present study's use of aggregate housing which has previously been shown to both increase body temperature (e.g. [5]) and to enhance hyperthermic response to *d*-amphetamine challenge [4]. Finally, as animals gain weight, surface-to-volume ratios decrease and this change mitigates against efficient heat exchange with the environment (e.g. [9,27]). It may therefore be that the heavier subjects experienced higher mortality rates as a function of less rapid heat dissipation leading to more severe states of hyperthermia. This hyperthermia hypothesis remains to be analyzed.

Alternative hypotheses to account for the present weight-PCA toxicity relation can be entertained. As subject selection was based solely on weight (within the given sex), it is likely that the 16 to 25-g animals were often younger than the 40 to 55-g subjects. Several studies have demonstrated alterations of various CNS neurotransmitter systems through ontogeny (e.g. [19]). Such changes have been discussed as explanations of altered toxicological and/or behavioral responses to drug challenge (e.g. [12, 13, 24]. The possibility that maturational changes in CNS neurotransmitter systems have mediated the present weight-toxicity effect deserves exploration. Similarly, prudence and the present data would suggest that appropriate weight controls be included in ontological studies of drug response.

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