Temperature-Dependent Toxicity of Adrenergic Agonists in Mice as a Basis for Treating *d*-Amphetamine Poisoning (37638)

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We have shown (1, 2) that pharmacologic responses to adrenergic agonists differed substantially when mice were exposed to high, room (about 23°) or low (4°) temperatures. Exposure to elevated ambient temperature increased the temperature responses to all adrenergic drugs tested while exposure to 4° decreased them. Moreover, salivation induced by adrenergic agonists was abolished in the cold, but was potentiated by temperatures only a few degrees above the usual room temperature (up to 30°). Salivation occurred spontaneously at temperatures above 32°.

These observations led us to compare the effects of room temperature and 4° on body temperature, salivation, and percentage deaths of mice receiving a considerably greater range of doses of adrenergic agonists than previously reported (1, 2). In this paper, the observations on salivation and temperature responses extend the dose-response curves previously reported to toxic and lethal doses. This study includes the effects of two indirectly acting sympathomimetic amines, *d*-amphetamine and *l*-ephedrine (not previously studied), and three catecholamines, *l*-norepinephrine, *l*-epinephrine, and *l*-isoproterenol. Our findings suggest a rational treatment for acute *d*-amphetamine poisoning, which is a clinical problem (3, 4), especially in children.

Materials and Methods. Male albino mice (NIH-GP strain, which was originally derived from a Swiss-Webster colony) were used. The mice were approximately one month old, weighed 17–22 g, and had access to food and water at all times. A group of 6 or 8 mice was housed in a plastic cage (42 × 24 × 16 cm, *i.e.*, 126 cm^2/mouse for 8 mice and 168 cm^2/mouse for 6 mice in a cage) with sawdust on the bottom. The cages were kept either at room temperature (about 23°) or at 4° in a walk-in cold room with a humidity of about 55%.

In some mice, colonic temperatures (T) were recorded with a Tele-Thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) before (T_0), one-half hour ($T_{½}$ hr), and 24 hr (T24 hr) after the administration of a drug. The thermocouple probe was inserted 3 cm into the rectum.

Salivation of mice was graded visually 0, 1, 2, and 3 as follows: 0, no signs of saliva; 1, corners of mouth and lower lip moist; 2, saliva covering both upper and lower lips and jaws; 3, armpits, chest as well as lips and jaws covered with saliva. The grade of salivation was recorded every 5 min for the first 30 min after the administration of a drug. The salivation response (S) is defined as the sum of the six successive gradings, with a maximum possible value of 18 (2, 5).

Deaths were recorded 0.5, 1, 2, 3, 4, 20, and 24 hr after the administration of drugs. The 0.5-hr, 4-hr, and 24-hr mortality data were evaluated with a computer by probit analysis (6). Where comparisons of LD_{50} values at 23° and 4° were made, parallel lines were fitted and then the statistic of interest was the difference of the log LD_{50} values, with its confidence range. The antilog of this difference, which is equal to the ratio of the LD_{50} values, was computed. If both the upper and lower 95% confidence limits were on

TABLE I. Protection Against *d*-Amphetamine Lethality by Transferring Mice to a Cold Room (4°).

Hr after *d*-amphetamine	Mice kept at room temperature (23°) (mg *d*-amphetamine/kg)			Mice moved to 4° 0.5 hr after *d*-amphetamine at 23° (mg *d*-amphetamine/kg)		
	10	20	40	20	40	60
2	2/8	2/8	0/8	0/8	0/7	0/8
4	6/8	4/8	4/8	0/8[a]	0/7[a]	0/8
24	6/8	5/8	5/8	0/8[a]	0/7[a]	0/8

[a] Significantly fewer deaths than in mice injected with the same dose and kept at room temperature throughout, $p < 0.05$, by Fisher exact test (7).

the same side of 1, then the ratio was considered statistically significant.

The data in Table I were analyzed for statistical significance by Fisher's exact test for 2×2 tables (7).

All drugs were injected intraperitoneally in a volume of 10 ml/kg (0.17–0.22 ml/mouse). Drugs were obtained from the following sources: *l*-epinephrine bitartrate, *l*-norepinephrine bitartrate, and *l*-isoproterenol-*d*-bitartrate dihydrate, Winthrop Labs., Special Chemical Department, New York, N. Y.; *l*-ephedrine sulfate, Merck & Co., Inc., Rahway, N. J.; *d*-amphetamine sulfate, K & K Labs., Plainview, N. Y. Doses of all drugs are expressed as milligrams of base per kilogram.

Results. Since indirectly acting adrenergic agonists are believed to produce their effects, at least in part, by the release of *l*-norepinephrine, we shall compare in this section the effects of *l*-norepinephrine, two other catecholamines, and *l*-ephedrine and *d*-amphetamine at both room temperature and at 4°.

Catecholamines. Figure 1 compares the 4-hr mortality data for *l*-norepinephrine and *l*-epinephrine at room temperature and at 4°. The LD_{50} values at room temperature were approximately twice those at 4° both at 4 hr and 24 hr. At 0.5 hr, the LD_{50} (at room T): LD_{50} (at 4°) value was 1.45 for *l*-norepinephrine and 1.46 for *l*-epinephrine. *l*-Isoproterenol was also more toxic at 4° than at room temperature. The ratio of LD_{50} values for *l*-isoproterenol at room temperature and 4° was 1.33 for 0.5-hr mortality. Nine of 16 mice receiving a dose of 300 mg/kg *l*-isoproterenol while being housed at 4° died within 4 hr, in contrast to no deaths in 4 or even 24 hr in 16 mice which received the same dose at room temperature.

Convulsions were not seen after the administration of any of the catecholamines at either temperature. At room temperature, the salivation seen with toxic doses of *l*-epinephrine and *l*-isoproterenol was less than that previously reported after lower doses of catecholamines (compare Fig. 2 in this paper with Fig. 3 in 5). Hyperthermia was produced at room temperature by doses of *l*-isoproterenol of 100 mg/kg or less but not by doses of 200–400 mg/kg (Fig. 2). Similarly, a dose of 3 mg/kg *l*-epinephrine produced a significant rise in body temperature but the larger dose of 5 mg/kg produced a significant fall (Fig. 2). At 4°, salivation did not occur after administration of any of the catecholamines. In the cold, doses of *l*-isoproterenol from 10–300 mg/kg produced dose-related falls in body temperature (Fig. 2).

l-Ephedrine and d-amphetamine. In contrast to the catecholamines, *l*-ephedrine and *d*-amphetamine were more toxic at room temperature than at 4° as shown by the fact that the ratios of LD_{50} (at room T): LD_{50} (at 4°) were less than 1 hr at 0.5 hr, 4 hr (Fig. 1), and 24 hr. It should be emphasized, however, that the ratio for *l*-ephedrine was closer to 1 than for *d*-amphetamine (0.73 *vs* 0.15 in Fig. 1). The slope of the dose-lethality line is steeper for *l*-ephedrine than for *d*-amphetamine. In fact, the slope for *l*-ephedrine is closer to those of *l*-norepinephrine and *l*-epinephrine than that of *d*-amphetamine. It should be noted that at both temperatures most doses of *d*-amphetamine produced either low or high mortality. However, since fitting

ADRENERGIC AGONIST	LD50 (room T) / LD50 (4° C.)	95% RANGE
l-Norepinephrine	2.17	1.37, 11.43
l-Epinephrine	1.92	1.29, 3.08
l-Ephedrine	0.73	0.58, 0.91
d-Amphetamine	0.15	0, 0.70

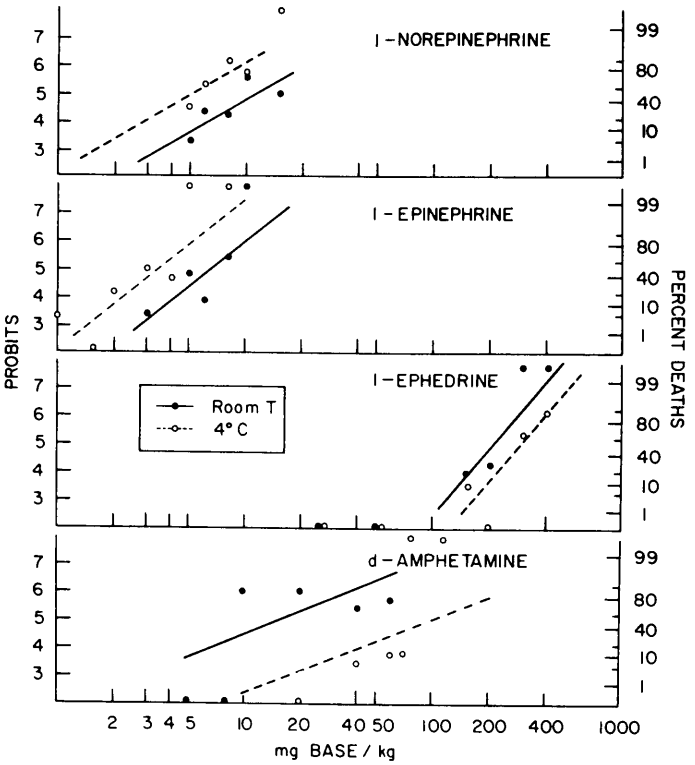

FIG. 1. Parallel lines fitted by probit analysis to the mortality data at room temperature and at 4° for *l*-norepinephrine, *l*-epinephrine, *l*-ephedrine, and *d*-amphetamine. The closed (room temperature) and open circles (4°) represent the percent deaths observed among 8–36 mice for each dose. The top of the figure shows the ratios of the LD_{50} (room T) and the LD_{50} (4°) values, with the 95% confidence limits for the ratio, which is statistically significant if the range does not include 1. The 0 and 100% mortality observed with some doses are plotted as 2 and 99% deaths, in order to show them on the graph.

parallel lines to the data gave finite confidence limits, the application of probit analysis was statistically valid.

Increased running was seen only after the lowest doses of *l*-ephedrine tested (25 and 50 mg/kg). Salivation was greater after 50 mg/kg of *l*-ephedrine at room temperature than after the other doses (Fig. 2). Doses of 200 mg/kg or greater of *l*-ephedrine failed to produce salivation even at room temperature. At 4°, salivation was not seen after any dose. The hyperthermic effect of *l*-ephedrine at room temperature paralleled the salivation response and the running activity. At 4°, doses of 150 mg/kg or greater produced sharp falls in body temperature.

At room temperature, *d*-amphetamine produced elevated body temperature and salivation at all doses tested, including lethal doses. Generalized tremors, clonus, and convulsions preceded death. Convulsions were not seen at 4°, but despite this low ambient temperature, there was no huddling; in fact, the animals ran rapidly around in the cages. Body temperatures were elevated at room temperature after all doses tested, while in the cold room

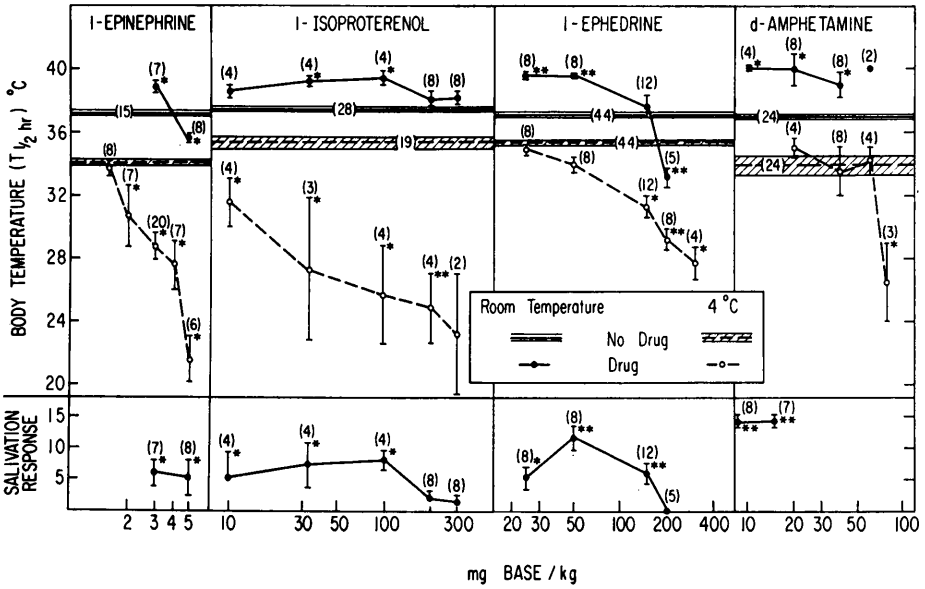

FIG. 2. A comparison of the body temperature and salivation responses to adrenergic agonists administered at room temperature (solid lines and circles) and 4° (dashed lines and open circles). Body temperature (T½ hr) was measured 0.5 hr after ip injection of an adrenergic agonist. Injections at 4° were made 3 hr after placing the mice in the cold room. Body temperatures immediately before administration of the agonists are represented by the horizontal solid line (room temperature) and dashed line (4°). The crosshatched areas and the vertical lines represent ± SE. The number of animals for each dose is given in parentheses. The salivation responses all represent observations made at room temperature, because no salivation occurred after any adrenergic agonist at 4°. An asterisk indicates the value differs significantly from the pre-injection value, $p < 0.05$ for a single asterisk and $p < 0.01$ for a double asterisk.

no dose of *d*-amphetamine produced hyperthermia or salivation, and the largest dose produced a marked fall in body temperature (Fig. 2). At room temperature, the larger doses (10 mg/kg or higher) did not produce the increased running activity seen at 4°.

Treatment of d-*amphetamine poisoning in mice by transfer to 4°.* Mice at room temperature were injected with various doses of *d*-amphetamine (Table I) and kept at room temperature for 30 min. During this time, tremors, twitches, and epileptiform convulsions had occurred in a few mice injected with 10 or 20 mg/kg and in all the mice injected with 40 or 60 mg/kg. One mouse, which received 40 mg/kg, died during this interval. All the mice salivated profusely. At 30 min, half of the mice which had received 20 and 40 mg/kg and all the mice which had received 60 mg/kg were transferred to a cold room (4°). None of the 23 animals transferred to the cold died during the 24-hr observation period. Upon removal from the cold to room temperature, the mice appeared to be normal, without signs of excitement, during an additional 24 hr before they were sacrificed. In contrast, 16 of the 24 mice kept at room temperature died within 24 hr. It is of special interest that all eight mice which had been injected with 60 mg/kg and transferred to 4° after 30 min survived, although we observed a mortality of 100% in the animals injected with this dose in the experiment designed for obtaining dose-lethality lines (Fig. 1).

Discussion. Our experiments showed that the catecholamines were more toxic at 4° while *l*-ephedrine and *d*-amphetamine were more toxic at room temperature. Other investigators have also found that *d*-amphetamine or *d,l*-amphetamine is less toxic at temperatures colder than 23° (8–10).

The increased toxicity of the catecholamines at 4° may be caused by the additive

effects of the injected amines with the circulating amines endogenously released by the cold stress activation of the sympathetic nervous system. Exposure to 4° would be expected to influence the toxicity of *l*-ephedrine and *d*-amphetamine in the same direction as that of *l*-norepinephrine, since these "indirectly-acting" sympathomimetic amines owe their pharmacologic effects at least in part to the release of norepinephrine. As shown by Dolfini *et al.* (11), who used slightly different experimental conditions, norepinephrine release is inhibited when the ambient temperature is 4°. These authors showed that depletion of brain norepinephrine by *d*-amphetamine (10 mg/kg, ip) was blocked if the mice were transferred to a cold room immediately after injection of *d*-amphetamine at room temperature.

Possible differences in absorption and distribution of *d*-amphetamine cannot explain the protective effect of the cold since some mice were injected with this amine at room temperature and kept there long enough for toxic signs to develop before being transferred into the cold room.

Aggregation of mice has been studied by many investigators as a factor responsible for increasing the toxicity of *d*-amphetamine (8, 9, 12–18). The area/mouse provided by most investigators was between 20–30 cm^2/mouse (13–16). Höhn and Lasagna (12) studied the effects of varying the area/mouse from 8.3–100 cm^2/mouse. They found a trend towards reduced toxicity of *d,l*-amphetamine with greater space per animal. In our experiments, the area per mouse was considerably greater (126 cm^2/mouse for 8 mice and 168 cm^2/mouse for 6 mice in a cage) so that the effects of aggregation were minimal. Under these conditions, the toxicity of *d*-amphetamine should approach that in isolated mice.

The toxicity of *l*-ephedrine and *d*-amphetamine at room temperature has been the subject of controversy (8–17). Several investigators (14, 16) have suggested hyperthermia and heat exhaustion as the cause of death. The epileptiform convulsions produced by these compounds may cause death by the inability of the vital centers to continue rhythmic functioning during their bombardment with proprioceptive impulses. Peterson *et al.* (19) have suggested that *d*-amphetamine causes death by a peripheral respiratory paralysis resulting from neuromuscular blockade. Either neuromuscular blockade, or posttictal depression subsequent to convulsive episodes, or both could explain the limpness and lack of activity observed with toxic doses of these amines at room temperature.

In the cold, hyperpyrexia, convulsions, and limpness did not occur after *l*-ephedrine or *d*-amphetamine. A reduction of toxicity of these amines in the cold would therefore be expected if the major factors responsible for death were not hyperpyrexia, convulsions, or neuromuscular blockade. It should be noted that curare-like drugs (20) and some convulsants (21) have been shown to be less toxic at low temperatures.

Although hypothermic drugs (*e.g.*, chlorpromazine, reserpine, propranolol) have been used in the experimental (14, 16, 22) and clinical (3, 4) treatment of *d*-amphetamine poisoning, we suggest the simpler and less complicated method of exposure to temperatures lower than room temperature. This proposal is based on the data in Table I. This treatment should have the advantage that possible complications arising from drug interactions are avoided.

This paper, in harmony with others (23, 24), illustrates that drugs evoke quantitatively and qualitatively different effects depending on the ambient temperature.

Summary. Exposure of mice to 4° abolished hyperthermia and salivation in response to the adrenergic agonists, *l*-norepinephrine, *l*-epinephrine, *l*-isoproterenol, *l*-ephedrine, and *d*-amphetamine. Comparisons of LD_{50} values at room temperature and 4° were made by fitting parallel lines to the mortality data by probit analysis. The catecholamines were more toxic at 4° than at room temperature, whereas mice injected with *l*-ephedrine or especially *d*-amphetamine were protected by exposure to cold. Mice injected with *d*-amphetamine at room temperature salivated profusely and convulsed, but the surviving animals were protected from death by transfer after 0.5 hr to 4°.

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