At the present time, the factors involved in the above points cannot be delineated to any greater extent. Regardless of the mechanistic aspect, the xanthine drugs do exhibit multiple solubility peaks as a function of the dielectric constant of the solvent system studied. The multiplicity of peaks is not limited to the above solutes. The dielectric solubility profiles for several antipyretic drugs such as acetanilid, *p*-methyl acetanilid, and *p*-ethoxy acetanilid (phenacetin) have been determined and are the subject of another communication. The aforementioned solutes also illustrate a multiplicity of dielectric requirements.

It may be advantageous to consider these dielectric requirements as being more representative of actual behavior relative to the solubility parameter concept. It is the authors' understanding that the solubility parameter concept apparently predicts only one value for solubility maximum. This occurs when the solubility parameter of the solvent mixture is greater or lesser than the value for the solute, the solubility is decreased, and therefore a solubility curve is observed. However, it should be noted that only one DR or solubility parameter may exist in which the observed solubility curve may supercede in magnitude all other subservient solubility curves. It can then be said that an optimum dielectric requirement or solubility parameter value may exist relative to the magnitude of solubility.

Occurrence of solubility peaks at the same value of the dielectric constant in solvent pairs other than dioxane–water should aid in lending some validity to the dielectric constant approach. Studies of this type will be reported in future communications.

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Some Neuropharmacological Properties of the Ephedrine Isomers

By GEORGE LANCIAULT and HAROLD H. WOLF

The central nervous system stimulating activity of the ephedrine isomers was compared with that of racemic amphetamine. Central nervous system alterations induced by acute administration of the drugs were evaluated by employing several standard techniques, including low-frequency electroshock and chemoshock threshold determinations, hexobarbital sleep-time alteration, and a behavioral rating scale. Evidence was obtained to show that the ephedrines vary in their ability to produce central stimulation. It was found that D(−)ephedrine and L(+)ephedrine were considerably more potent than D(−)pseudoephedrine and L(+)pseudoephedrine.

Previous work with the ephedrine isomers involving the cardiovascular system has demonstrated that these compounds vary markedly in their effects on this system (1, 2). D(−)Pseudoephedrine is reported to lack the ability to produce a typical ephedrine pressor response, causing instead a depression of blood pressure. This isomer also has been observed to produce vasodilation in vascular beds of dogs, in contrast to D(−)ephedrine which produces vasoconstriction. Furthermore, renal and vertebral arterial blood flow in the dog decreases when L(+)ephedrine or L(+)pseudoephedrine are given, whereas with D(−)ephedrine the flow increases.

Although there are many literature references relative to D(−)ephedrine and its effects on the central nervous system (3–6), reports of work with the other isomers are scanty and inconclusive. However, some quantitative differences in the central activity of these compounds have been reported. For example, Trevan (3) has demonstrated that the isomers vary considerably in their ability to act as analeptics in anesthetized

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mice, and Schulte *et al.* (7), using a work adder technique, have reported that D(−)ephedrine is a more potent central nervous system stimulant than either of the pseudoephedrines. In contrast, Schoot *et al.* (8) have reported that the difference between D(−)ephedrine and L(+)pseudoephedrine is slight with respect to central stimulant effects.

In view of the variable effects of these isomers on the periphery and the relative paucity of information pertaining to their central activity, it was felt that a critical evaluation of the central stimulating activity of these compounds, as compared with that of racemic amphetamine, was warranted. The results obtained constitute the basis of this report.

EXPERIMENTAL

General Procedures.—Swiss albino, random bred, male mice with a weight range from 15 to 28 Gm. (Maxfield Animal Supply, Cincinnati, Ohio) were used throughout all the experiments. They were maintained on rat chow (Purina) and allowed free access to food and water except during actual experimentation. The compounds investigated were D(−)ephedrine, D(−)pseudoephedrine, L(+)ephedrine, L(+)pseudoephedrine, and racemic amphetamine.

All drugs were administered intraperitoneally in aqueous solution, the ephedrines as their hydrochloride salts and amphetamine as its sulfate. All tests were conducted at the time of peak effect of each drug.

Determination of the Stimulating Dose$_{50}$ (SD_{50}).—The SD_{50}s of the test compounds were determined by the utilization of a rating scale. Various workers (7, 9, 10) have described methods of rating central nervous system stimulating activity, but none of these were ideally suited to the work involved. Thus, a central nervous system activity rating scale was specifically devised for this study.

An arbitrary dose of 50 mg./Kg. of each drug was given to ten mice, while ten more received a requisite volume of saline. The animals were examined for the following responses:

A.—Piloerection.

B.—Hyperirritability—the manner in which a mouse reacts to a blast of air (5 ml.) from a syringe with a 23-gauge needle. A positive response consists of a jumping reaction, sometimes even a somersault, from one side of the cage to the other. When a control mouse is exposed to the blast, the most common reaction is closing of the eyes or turning back of the ears.

C.—Increased activity—a greater spontaneous motor movement shown by the treated animal as compared with controls.[1]

[1] Increased activity was determined by placing each animal singly into an activity cage (dimensions 2 × 2 × 1 ft.). The floor and sides were marked off into 3-in. sq. grids. The animal was free to move anywhere in the cage, and the number of squares crossed per minute was noted. The counts for the controls were averaged and the standard error calculated. The mean and standard error of the controls were compared with each individual score of the drug-treated animals. If the activity score of the drug-treated mouse was greater than the saline controls (mean and standard error), it was considered a positive response.

D.—Tremors—involuntary movements of the limbs resembling a shaking motion.

E.—Jerks—sudden reflex movements. This response is seen when the animal starts to take a step and suddenly brings the limb back toward the body, producing an uncoordinated movement.

F.—Clonic convulsions—a state in which the mouse exhibits a brief burst of spontaneous alternating flexion and extension of either fore or hind limbs.

G.—Tonic convulsions—a state of persistent contraction of the flexor or extensor muscles of the fore or hind limbs.

H.—Loss of righting reflex—a condition in which the equilibrium cannot be maintained. Righting is impossible when the animal is placed on its back.

I.—Death.

Each response was given a numerical value for scoring purposes. The reaction which occurred most frequently (piloerection) at low doses was given the value of 1. The responses which appeared more frequently at higher doses (hyperirritability, increased activity) were then given succeeding values of 2, 3, etc.

Any of the above reactions observed in an animal were recorded as positive responses (Table I). The numerical values of the noted responses for each animal were totaled for several time intervals (10, 30, 45, 60, and 90 min.) to determine the degree of stimulation as a function of time.

TABLE I.—SAMPLE SCORING SHEET FOR CENTRAL STIMULANT ACTIVITY[a]

Response	Numerical Value (See Text)	Time, min. 10	30	45	60	90
Death	9	..	..	..	..	..
Loss of righting reflex	8	..	..	..	..	..
Convulsions, tonic	7	..	..	..	..	..
Convulsions, clonic	6	..	..	..	..	..
Jerks	5	..	..	..	..	..
Tremors	4	..	..	..	..	..
Increased activity	3	−	+	+	+	+
Hyperirritability	2	−	+	+	−	−
Piloerection	1	+	+	+	+	+
Total		1	6	6	4	4

[a] Drug, D(−)ephedrine; concentration of drug, 1.25%; strain, random-bred, Swiss albino, male mouse; solvent, water; dose, 125 mg/Kg.; route, intraperitoneally.

TABLE II.—SD_{50} AND TIME OF PEAK ACTIVITY OF THE TEST COMPOUNDS

Drug	SD_{50}, mg./Kg.		Time of Peak Drug Effect, min.
D(−)Ephedrine	110	(75.8–159.0)[a]	45
D(−)Pseudoephedrine	160	(96.9–264.0)	60
L(+)Ephedrine	181	(157.0–208.0)	30
L(+)Pseudoephedrine	150	(115.0–195.0)	10
Racemic amphetamine	54	(40.0– 72.9)	45

[a] 95% confidence limits.

To establish a base level of stimulation, an arbitrary total value of 5 was taken as evidence of drug-induced increased central activity. Any animal receiving a total score of 5 or greater was considered to be stimulated by the drug involved.

The scoring procedure was then repeated with increasing doses of the compound, in groups of ten mice each, until at least three dose levels were obtained which produced between 0 and 100% stimulation. The SD_{50} was calculated by the method of Litchfield and Wilcoxon (11).

A qualitative comparison was conducted to determine whether the quantitative results obtained from the rating scale yielded doses of the test compounds which were equipotent from the standpoint of observed behavior. In this procedure the SD_{50} of each drug was given to a group of ten animals. At the time of peak effect, the behavior of each animal was rated by means of the scale, and average scores were compared by means of a Kruskal-Wallis analysis of variance (12).

Threshold Studies.—Low-frequency electroshock seizure threshold (l.f.E.S.T.) and pentylenetetrazol[2] seizure threshold were determined. Except that a Grass stimulator (S-4-G) was used for the determination of l.f.E.S.T., the details of the electroshock test and the characteristics of the apparatus have been described elsewhere (13–15).

Sixty-four mice were randomly divided into two groups of 32 animals each. One group was given the drug to be tested (SD_{50}) and the other the requisite volume of saline. At the time of peak drug effect, the l.f.E.S.T. of both groups was determined as previously described (16). The threshold ratio (threshold of drug group/threshold of control group) was calculated. Threshold ratios were determined by this procedure for the SD_{50} of each drug. Thus, for this determination, 320 mice were employed.

The experimental design for the pentylenetetrazol test required the use of 50 drug-treated and 50 saline-treated mice. Groups of ten animals each were given either drug (SD_{50}) or saline and evaluated for pentylenetetrazol seizure threshold by the technique of Orloff (17). The amount of pentylenetetrazol required to produce clonus was calculated for each group and the results compared by means of a factorial analysis of variance.

Hexobarbital Potentiation.—Sixty mice were randomly divided into six groups of ten mice each. Ten animals were given a requisite volume of saline, while the remaining groups received an SD_{50} of the test compounds. At the time of peak drug effect, sodium hexobarbital (100 mg./Kg.) was given intravenously. The sleeping time in minutes was measured from the end of injection until righting reflex was regained (18). Average sleeping times were compared statistically by means of group comparison t tests.

RESULTS

The results obtained from the central nervous system rating scale studies (Table II) show that the SD_{50}s of the ephedrine isomers do not manifest any significant differences among themselves while showing a statistically significant difference when compared with racemic amphetamine. An examination of this table reveals that the isomers are only 29 to 49% as potent (on a mg./Kg. basis) as amphetamine in inducing gross behavioral changes. As also can be seen from the table, the times of peak activity varied from drug to drug ranging from 10 min. for L(+)pseudoephedrine to 60 min. for D(−)pseudoephedrine.

From the qualitative comparison, as previously described, an analysis of variance yielded results showing the SD_{50}s of the investigational drugs to be equipotent. Thus, although the average grades of gross stimulation observed with the various SD_{50}s were not exactly the same, the confidence limits overlapped at the 0.05 level of significance.

In both the electroshock and chemoshock studies, D(−)ephedrine and L(+)ephedrine were more potent in their abilities to lower seizure threshold than were either of the pseudoephedrines (Figs. 1 and 2).

It is evident from the data depicted in Fig. 1 that the SD_{50} of all agents investigated had a marked lowering effect on l.f.E.S.T. It may also be observed that D(−)ephedrine and L(+) ephedrine are more effective than either of the pseudophedrines. Racemic amphetamine, although it significantly lowered threshold, was least potent by this test.

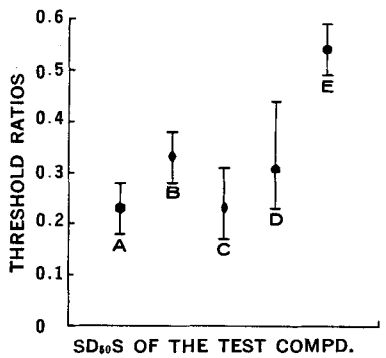

Fig. 1.—l.f.E.S.T. ratios of the compounds tested (CC50 of drug-treated/CC50 of saline-treated). Key: *A*, D(−)ephedrine; *B*, D(−)pseudoephedrine; *C*, L(+)ephedrine; *D*, L(+)pseudoephedrine; *E*, racemic amphetamine ($p = 0.05$).

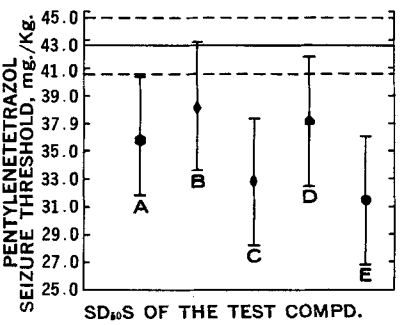

Fig. 2.—Pentylenetetrazol seizure thresholds of the tested compounds. Key: *A*, D(−)ephedrine; *B*, D(−)pseudoephedrine; *C*, L(+)ephedrine; *D*, L(+)pseudoephedrine; *E*, racemic amphetamine, ⚌⚌⚌⚌, saline controls ($p = 0.05$).

[2] Marketed as Metrazol.

The results of another threshold experiment, in which the timed intravenous pentylenetetrazol infusion technique was used to measure the level of central nervous system excitability are portrayed in Fig. 2. It can be seen that the SD_{50} of D(−) ephedrine, L(+)ephedrine, and racemic amphetamine significantly lowered pentylenetetrazol threshold by 18, 23, and 26%, respectively, when compared with saline-treated controls. In contrast, D(−)pseudoephedrine lowered threshold by only 10% (not statistically significant), and L(+)pseudoephedrine lowered threshold by 13% (borderline significance).

The doses of the compounds investigated had no consistent effect on hexobarbital sleeping time which would permit a valid generalization to be drawn.

DISCUSSION

The data presented indicate that the profiles of neuropharmacological activity of the ephedrine isomers are qualitatively similar to each other as well as to racemic amphetamine. Thus, all compounds investigated produce similar dose-dependent changes in the gross behavior of the intact mouse. For example, low doses of all drugs cause piloerection, hyperirritability, and increased motor activity, whereas high doses induce tremors, clonic and tonic convulsions, loss of righting reflex, and acute respiratory failure leading to death. Furthermore, all compounds tend to lower the threshold for electrical and chemical-induced convulsions.

However, it is also apparent from the data that the compounds differ considerably in their quantitative activity in the central nervous system. Thus, with the exception of the l.f.E.S.T. test, racemic amphetamine manifests consistently greater stimulant activity than do any of the ephedrines. Moreover, the degree of central stimulation produced by D(−)pseudoephedrine and L(+)pseudoephedrine is considerably less than that produced by D(−)ephedrine and L(+)ephedrine.

These findings help to explain the results of some previous work (19) in which it was demonstrated that these drugs varied considerably in their ability to produce the well-known "aggregation" phenomenon (20). In this study an examination of lethality potency ratios (LD_{50} isolation/LD_{50} aggregation) revealed that amphetamine was five to eight times more potent than the ephedrine isomers in the expression of this phenomenon. Furthermore, while both D(−)ephedrine and L(+)ephedrine were significantly more lethal to grouped *versus* isolated mice, L(+)pseudoephedrine did not demonstrate this phenomenon. Although the fourth isomer, D(−)pseudoephedrine, did manifest increased toxicity in the aggregated environment, the magnitude of this effect was less than that observed with D(−)ephedrine and L(+)ephedrine. Thus, it would appear that the results obtained in these earlier studies simply reflect the underlying ability of the drugs investigated to induce varying degrees of central stimulation.

The reasons for the quantitative differences in central activity exhibited by the four ephedrine isomers remain obscure. Recently, a considerable amount of evidence has been uncovered indicating that central receptors for catecholamines, *e.g.*, norepinephrine and dopamine, are involved in the action of psychomotor stimulants. Such drugs may act directly on central catecholamine receptors and thus mimic the action of naturally occurring substances (21, 22) or act indirectly by causing an increase in the catecholamine concentration near the receptors (23). Although evidence based on peripheral effects indicates that D-configuration of the β-carbon in the ephedrines favors direct action while L-configuration favors indirect action (24), no such rigid structure-activity relationship is yet apparent for the central activity of these compounds. It may be that the quantitative differences in activity observed between the ephedrines and pseudoephedrines reflect differences in physical properties of the diastereoisomers, *e.g.*, solubility in biological membranes and penetration of the blood-brain barrier, which could then regulate the ease by which these substances gain access to central receptors.

It is anticipated that an examination of the above factors together with an evaluation of the ability of these agents to alter levels of brain amines may help to elucidate their mechanism of stimulant activity. Such studies are currently in progress.

SUMMARY

Although all four ephedrine isomers manifest overt stimulation of the central nervous system, the magnitude of this activity is considerably less than that of amphetamine.

The enantiomers D(−)ephedrine and L(+) ephedrine demonstrate considerably more central activity than do the corresponding pseudoephedrine enantiomers.

This system of stereoisomers may provide a useful paradigm for further investigation into structure-action relationships in the central nervous system.

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