Effects of *dl*-, *d*-, and *l*-Amphetamine on Levarterenol Tachyphylaxis in the Isolated Heart of *Venus mercenaria*

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The comparative potencies of *dl*-, *d*-, and *l*-amphetamine in completely blocking the onset of levarterenol tachyphylaxis (characterized by diminished negative inotropic responses) in the isolated heart of *Venus mercenaria* were as follows: *d*-amphetamine was the most potent; *dl*-amphetamine and *l*-amphetamine were equally active, and less potent than the *d*-isomer. The observance of positive inotropic responses, occurring with higher doses of amphetamine isomers, indicated that both agents may act at the same receptor site. Furthermore, it is speculated that amphetamine may possibly block the inhibitory cardiac sites upon which levarterenol acts, thereby allowing the latter agent to exert a stimulatory action.

THE TERM tachyphylaxis or skeptophylaxis is used to denote a diminution in the response to successive equal doses of a pharmacological agent within a limited time interval. The diminished pressor responses which occur after consecutive doses of ephedrine (1), and the inability of repeated administrations of atropine to depress intestinal motility (2), are well-known examples of this phenomenon.

In 1953 Welsh (3), while evaluating the effects of various drugs on the isolated heart of the quahog—*Venus mercenaria*—observed the development of acute tolerance to repeated administrations of epinephrine. Fujita and Mann (4) reported that tachyphylaxis, characterized by diminished negative inotropic responses, occurred in the *Venus* heart after three or four successive doses of levarterenol. Further studies (5) showed that this response could be partially or completely blocked by ephedrine hydrochloride, *l*-ephedrine being more potent than either the *d*-isomer or the racemic mixture in this respect.

Amphetamine, a sympathomimetic agent chemically related to ephedrine, is believed to exert its peripheral actions in a manner similar to that of ephedrine, *i.e.*, adrenergic blockade (6). Because it had been noted that *l*-amphetamine was more potent than *d*-amphetamine in pressor activity (7), it was the purpose of this investigation to determine whether or not amphetamine would act as ephedrine in this situation and which isomer was the more potent.

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EXPERIMENTAL

The bathing chamber for the isolated heart preparation was constructed of plexiglas and consisted of a section of tubing 4.5 cm. in diameter and 5 cm. in length with a circular base enclosing one end. A hole in the center of the base provided for the insertion of a hypodermic syringe sleeve through which was passed a ground-glass syringe plunger. A small fish hook was fused into the upper end of the plunger. By raising the glass rod vertically until it protruded above the lip of the chamber, the heart could easily be attached to the hook, while lowering the rod immersed the heart in the bathing medium (filtered sea water), thereby minimizing handling and stretching. Inflow and outflow of sea water were accomplished by means of tubes, one of which provided for aeration, inserted through the base of the chamber which was graduated at a volume of 40 ml.

The heart of *Venus mercenaria* was isolated according to the methods of Welsh and Taub (8), and Wait (9). Contractions were recorded on a Livingston long-paper kymograph. The specific gravity of the sea water was maintained at 1.025 and the temperature was kept at 24–26°.

The tabulation of results was done in accordance with the method of Ciuchta and Mann (5). The figures in Table I, representing per cent inhibition, were derived by dividing the lowest amplitude value (in millimeters) recorded within three minutes after the administration of levarterenol, by the amplitude value preceding the administration of the drug. The terms trial I, trial II, and trial III pertain to the three types of treatment that each *Venus* heart underwent. Trial I refers to the administration of three successive doses of levarterenol to establish a tachyphylactic response in a fresh heart; trial II designates that the same heart, after washing, was pretreated with the appropriate isomer and dose of amphetamine sulfate (0.1%) before the subsequent injection of three doses of levarterenol; and trial III refers to the re-establishment of tachyphylaxis by the administration of three successive doses of levarterenol to the heart after washing at the completion of trial II.

Effect of *dl*-Amphetamine Sulfate on Levarterenol Tachyphylaxis.—With the *Venus* heart suspended in the bathing chamber containing 40 ml. of sea

TABLE I.—PER CENT INHIBITION OF THE PREINJECTION AMPLITUDE OF THE HEART OF *Venus mercenaria* AFTER EACH SUCCESSIVE ADMINISTRATION OF 0.40 ML. OF LEVARTERENOL

No.	Trial I — Dose of Levarterenol 1st	2nd	3rd	Trial II — Dose of Levarterenol 1st	2nd	3rd	Trial III — Dose of Levarterenol 1st	2nd	3rd
Trial II Includes *dl*-Amphetamine Sulfate Pretreatment (0.80 mg./40 ml. Sea Water) Prior to Levarterenol									
1	30	21	0	0	12	5	38	30	12
2	0	10	10	0	0	0	35	21	0
3	42	20	0	0	0	0	0	20	59
4	17	8	0	0	0	0	0	18	6
5	49	23	0	0	0	0	38	15	14
6	19	0	0	0	0	0	0	6	0
7	44	16	0	0	0	0	65	5	0
8	15	0	0	0	0	0	7	5	0
9	24	11	0	0	0	0	19	62	46
10	22	19	0	0	0	0	0	0	0
11	40	15	0	0	0	0	23	36	0
12	9	9	0	0	0	0	7	12	0
13	19	33	7	0	0	0	6	9	6
Trial II Includes *d*-Amphetamine Sulfate Pretreatment (0.50 mg./40 ml. Sea Water) Prior to Levarterenol									
1	39	25	0	8	7	0	27	24	0
2	16	7	5	0	0	0	7	9	0
3	100	76	11	0	0	0	5	6	0
4	50	21	0	0	0	0	11	10	0
5	5	15	8	0	0	0	0	0	0
6	35	15	0	0	0	0	7	19	6
7	22	6	0	0	0	0	7	5	0
8	17	14	5	0	0	0	9	5	0
Trial II Includes *l*-Amphetamine Sulfate Pretreatment (0.80 mg./40 ml. Sea Water) Prior to Levarterenol									
1	32	23	11	15	0	0	19	5	0
2	60	27	0	14	0	0	26	13	0
3	69	42	0	0	0	0	44	16	0
4	25	12	0	0	0	0	0	7	0
5	47	0	5	0	0	0	13	5	0
6	16	0	0	0	0	0	6	7	0
7	26	11	0	0	0	0	13	6	0
8	58	28	0	0	0	0	7	10	0
9	25	8	0	0	0	0	8	0	0
10	41	0	0	0	0	0	24	11	0
11	27	25	10	0	0	0	51	41	0
12	19	10	0	0	0	0	0	0	0
13	18	17	0	0	0	0	0	5	0
14	23	10	5	0	0	0	12	37	0
15	34	13	0	0	0	0	25	9	0

water, normal contractions were recorded, whereupon the ventricle was challenged with three 0.40-ml. doses of levarterenol[1] administered at 6-minute intervals to elicit tachyphylaxis. The majority of hearts were unresponsive to the third dose of levarterenol, while several preparations reacted slightly. Nevertheless, the pattern of diminishing responses to the three successive injections indicated that tachyphylaxis had occurred. The concentrations of levarterenol in 40 ml. of sea water after each dose were as follows: 0.4 mg. (1×10^{-5}) after the initial dose, 0.8 mg. (2×10^{-5}) after the second, and 1.2 mg. (3×10^{-5}) after the third. Following the development of the tachyphylactic pattern, the ventricle was allowed to beat for three minutes, then it was washed and rewashed with fresh sea water for one-minute periods. This procedure terminated trial I.

After the wash, the heart was again permitted to beat for approximately three minutes, or until it had resumed normal contractions. It was next treated with a dose of *dl*-amphetamine sulfate (0.40, 0.50, 0.70, or 0.80 mg.) so that when injected into 40 ml. of sea water the concentrations were respectively: 1.0×10^{-5}, 1.25×10^{-5}, 1.75×10^{-5}, or 2.0×10^{-5}. Approximately four minutes later, the heart was treated with three 0.40-ml. doses of levarterenol. This phase of the experiment was referred to as trial II.

Trial III consisted of the administration of three 0.40-ml. doses of levarterenol for the purpose of re-establishing tachyphylaxis in the same heart subsequent to final washing after trial II, and after normal contractions had been obtained.

Pretreatment with the lowest dose (0.4 mg.) of *dl*-amphetamine caused a complete blockage to subsequent doses of levarterenol in 4 out of 10 cases; 0.5 mg. completely blocked only 1 out of 10 cases; 0.7 mg. inhibited tachyphylaxis in 5 out of 8 cases; 0.8 mg. caused a complete blockage of the negative inotropic response in 12 out of 13 determinations (Table I).

Effect of *d*-Amphetamine Sulfate on Levarterenol Tachyphylaxis.—The heart was treated as in the previous part, with the exception of trial II which consisted of pretreatment with a dose of *d*-amphetamine sulfate (0.40, 0.50, 0.60, 0.70, or 0.80 mg.) so that the bath concentrations attained were: 1.0×10^{-5}, 1.25×10^{-5}, 1.50×10^{-5}, 1.75×10^{-5}, or 2.0×10^{-5}.

[1] Levophed bitartrate, 0.1% base, Winthrop Laboratories.

Pretreatment with the 0.40-mg. dose of *d*-amphetamine caused a complete blockage to the negative inotropic response of levarterenol in 3 out of 8 cases. The five remaining hearts were partially blocked as shown by a great diminution of response as compared to trial I. A dose of 0.5 mg. caused 7 out of 8 hearts to be unresponsive to all doses of levarterenol. Nine hearts were treated with either 0.60, 0.70, or 0.80 mg. of *d*-amphetamine (3 at each dose) with the following results: all 9 hearts showed a complete blockage to subsequent doses of levarterenol; in trial III, after thorough washing, 6 of the 9 hearts remained either completely refractory to all doses of levarterenol or partially responsive to only one dose.

Effect of *l*-Amphetamine Sulfate on Levarterenol Tachyphylaxis.—The heart was treated as previously described with the exception of trial II which consisted of pretreatment with a dose of *l*-amphetamine sulfate (0.40, 0.60, 0.70, or 0.80 mg.) to attain bath concentrations of 1.0×10^{-5}, 1.5×10^{-5}, 1.75×10^{-5}, or 2.0×10^{-5}. The 0.40 and 0.60 mg. doses produced complete blockage of levarterenol tachyphylaxis in 1 out of 5 and 1 out of 6 cases, respectively; 2 out of 7 hearts were blocked by 0.70 mg.; and complete blockage of 13 out of 15 hearts occurred at the 0.80 mg. dosage level.

DISCUSSION

The results of trial I have reconfirmed the published observations of Fujita and Mann (4) that tachyphylaxis to the negative inotropic response of levarterenol occurs in the isolated *Venus* heart. In trial II, pretreatment with the isomers of amphetamine sulfate partially or completely blocked this effect in a manner similar to that produced by the isomers of ephedrine hydrochloride (5). The latter study revealed that *l*-ephedrine was the most potent inhibitor of the tachyphylactic response. This investigation has shown that *d*-amphetamine possesses the greatest blocking activity, while *l*-amphetamine and the racemic mixture are approximately equal in their ability to block the tachyphylactic response. The comparative potencies of these isomers are recorded in Table II. From these results, it is reasonable to assume that the spatial configurations of the amphetamine and ephedrine molecules are implicated in the antagonism of levarterenol.

The theory of Gaddum and Kwiatowski (10), which attempted to explain the mechanism of action of ephedrine, has also been applied to amphetamine and offers a possible explanation for the observed effects in the *Venus* heart. Accordingly, amphetamine might exert its antagonistic actions upon the same receptor mechanism which levarterenol activates, *i.e.*, by combining with levarterenol receptors in a manner comparable to substrate competition, thus preventing the onset of acute tolerance. Another similarity between amphetamine and ephedrine was noted particularly at higher dosage levels. When the hearts were pretreated with amphetamine in trial II, positive inotropic effects were frequently seen which were potentiated by subsequent doses of levarterenol, perhaps indicating that both agents may act at mutual receptor sites. Consequently, large doses of amphetamine might block the inhibitory sites and allow levarterenol to produce its positive inotropic action upon excitatory receptors.

The pretreatment of *Venus* hearts with large doses (0.5 mg. plus) of *d*-amphetamine in trial II frequently resulted in a failure to re-establish the tachyphylactic response in trial III, despite several thorough washings. This effect was seldom encountered when *l*-amphetamine was used, but occasionally occurred after large doses of the racemic mixture. It may be due to a binding of the *d*-isomer at the receptor site to prevent the characteristic levarterenol response, a phenomenon which further supports the contention that *d*-amphetamine is the most potent isomer in abolishing levarterenol tachyphylaxis.

SUMMARY

Pretreatment of the isolated *Venus* heart in trial II with various concentrations of the *dl*-, *d*-, and *l*-isomers of amphetamine has indicated that *d*-amphetamine is the most potent inhibitor of levarterenol tachyphylaxis, while *dl*-amphetamine and *l*-amphetamine, although equally active, are less potent than the *d*-isomer.

It is postulated that amphetamine could block levarterenol responses by acting on the same receptor sites which levarterenol affects.

Observance of a positive inotropic effect with higher doses of amphetamine isomers, which is proportional to their relative blocking abilities, and the potentiation of this response with subsequent administrations of levarterenol indicated that both agents may act at the same receptor site. Furthermore, it is presumed that amphetamine may block the inhibitory sites, thereby allowing levarterenol to exert a stimulatory action.

TABLE II.—PERCENTAGE OF *Venus* HEARTS EXHIBITING COMPLETE BLOCKAGE TO ALL ADMINISTRATIONS OF LEVARTERENOL AFTER PRETREATMENT WITH VARIOUS DOSES OF *dl*-, *d*-, AND *l*-AMPHETAMINE SULFATE IN TRIAL II

dl-Amphetamine		*d*-Amphetamine		*l*-Amphetamine	
Dose[a]	% Unresponsive Hearts	Dose[a]	% Unresponsive Hearts	Dose[a]	% Unresponsive Hearts
0.40	40 (4/10)	0.40	38 (3/8)	0.40	20 (1/5)
0.50	10 (1/10)	0.50	88 (7/8)	0.60	17 (1/6)
0.70	63 (5/8)	0.60	100 (3/3)	0.70	29 (2/7)
0.80	92 (12/13)	0.70	100 (3/3)	0.80	87 (13/15)
		0.80	100 (3/3)		

[a] Dose is mg./40 ml. sea water.

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Effect of Ultrasonic Waves on the Stability of Selected Surface-Active Agents, Sulfonamides, and *p*-Aminobenzoic Acid

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Data are presented showing the effect of ultrasonic waves on the stability of certain surface-active agents, sulfonamides, and *p*-aminobenzoic acid. Under the experimental conditions employed, the surface-active agents were stable and the breakdown of the sulfonamides and *p*-aminobenzoic acid was greater than could be accounted for on the basis of peroxide formation.

BEAL and Skauen have reported that the viscosity of several surface-active agents is reduced upon exposure to ultrasonic waves (1). This suggests the possibility of a decrease in molecular weight of these agents as a result of depolymerization or some other type of chemical decomposition. Similar breakdown of large molecules has been reported by several authors (2–5). Araujo has reported the decomposition of procaine penicillin G and sulfathiazole in an ultrasonic field (6). The authors have noted similar decomposition of procaine and buteth-amine hydrochlorides. This decomposition was apparently of an oxidative nature and was prevented by the use of sodium bisulfite as an antioxidant (7). A number of authors have reported the formation of peroxides in aqueous solutions as a result of treatment with ultrasonic waves (8–11). In view of these investigations, it was deemed to be of value to determine if surface-active agents are stable in an ultrasonic field and if peroxide formation is responsible for the breakdown of sulfonamides and similar compounds. This communication reports the effect of ultrasonic waves at a frequency of 400 kilocycles per second upon the stability of selected surface-active agents. This should give an indication as to the advisability of using these agents when preparing emulsions or suspensions by ultrasonics. The amount of peroxides formed as a result of exposure to ultrasonic waves of this frequency was determined. The effect of similar amounts of peroxide alone on the stability of one of the sulfonamides was compared with the effect of ultrasonic waves upon stability. This should indicate if the breakdown due to ultrasonic waves is caused primarily by the formation of peroxides.

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EXPERIMENTAL

Calibration of the Generator.—The ultrasonic generator employed in this study was constructed at Purdue University and was designed for use with a barium titanate transducer. The generator is rated at 250 watts with a variable frequency range. A Hypersonic Transducer, model BU-305,[1] with a focused bowl of barium titanate was employed. The generator was calibrated by determining the wattage produced at various rheostat settings by multiplying the power amplifier voltage and the power amplifier current as shown by appropriate meters on the generator. All of the experiments involving insonation were conducted at a frequency of 400 kilocycles per second and an energy level of approximately 150 watts.

Stability of Selected Surface-Active Agents.—The surface-active agents chosen for this part of the study were Myrj 45,[2] G-2159,[2] Aerosol AY,[3] Tween 80,[2] Span 80,[2] and Triton X-200.[4] Solutions of these surfactants were insonated for extended periods of time. The hydroxyl and saponification

[1] Marketed by Brush Development Co., Cleveland, Ohio.
[2] Marketed by Atlas Powder Co., Inc.
[3] Marketed by American Cyanamid Co., Inc.
[4] Marketed by Rohm and Haas Co. Inc.