EFFECT OF ULTRA-VIOLET IRRADIATION OF PHENYLEPHRINE SOLUTIONS

BY F. P. LUDUENA, ANN L. SNYDER, AND A. M. LANDS

From the Sterling-Winthrop Research Institute, Rensselaer, N.Y.

Received February 15, 1963

Solutions of phenylephrine in distilled water were irradiated with ultra-violet light for 3 hr. The irradiated solutions tested on perfused guinea-pig lungs had a bronchodilator potency greater than that of the original non-irradiated phenylephrine solution. The bioassay and photofluorometric determinations (Shore and Olin method) suggested that adrenaline had been formed from phenylephrine in the irradiated solution.

As early as 1931, Ewing, Blickensdorfer and McGuigan reported that the pressor activity of aqueous solutions of 1-(4-hydroxyphenyl)-2-methylaminoethanol (oxedrine, Sympatol) was increased by ultra-violet irradiation, whereas the same treatment reduced the pressor activity of adrenaline solutions and made solutions of ephedrine hypotensive. Inactivation of adrenaline by ultra-violet irradiation had been reported earlier that year by Verda, Keer and Burge (1931). Konzett and Weis (1938, 1939) confirmed the results obtained with adrenaline and by biological and chemical tests were able to confirm also the formation of adrenaline in irradiated solutions of oxedrine.

The increase in activity produced by irradiation suggested the possibility that oxedrine had been changed to adrenaline. After irradiation, the adrenaline solutions were discoloured and a similar discoloration was seen after irradiation of oxedrine. This suggested that catechol formation may be one of the intermediate steps in the process of discoloration of phenolic sympathomimetic amines solutions induced by ageing. This possibility is of interest since West and Whittet (1960) found that 10 per cent solutions of phenylephrine [1-(3-hydroxyphenyl)-2-methylaminoethanol] stored at room temperature in amber-coloured bottles "became yellow or pink within a few weeks of issue", even when they contained sodium metabisulphite (0·1–0·2 per cent). When pronounced discoloration occurred after storage in colourless bottles, from prolonged storage or after the addition of hydrogen peroxide to the solution, the loss in pharmacological activity was minimal (pressor effect on anaesthetised rats or cats). West and Whittet also found no direct relation between loss of activity and amount of discoloration.

That oxidation resulted in discoloration of the solutions suggested the possibility that adrenaline may have been formed as a first step in this process and that the small amounts formed could not have been detected in the presence of large amounts of phenylephrine by the bioassay method used. To test for the possible formation of adrenaline by oxidation, we subjected solutions of phenylephrine to ultra-violet irradiation and estimated the adrenaline content by bronchodilator activity and by chemical analysis.

METHODS

Solutions of phenylephrine hydrochloride in distilled water (25–50 ml.) were placed in Petri dishes (3·5 in. diameter and 0·5 in. depth) on an adjustable platform under an ultra-violet lamp (Analytic Model Quartz Lamp, Engelhard Hanovia, Inc., Newark, N.J.). The solutions, placed approximately 23 cm. below the burner and 3·5 cm. below the Hanovia SC-5028 heat filter, were irradiated for 3 hr., after which they had the colour of a strong tea infusion. Distilled water was added to replace that lost by evaporation.

The solutions were tested on the perfused guinea-pig lung preparation (Sollmann and von Oettingen, 1928; Tainter, Pedden and James, 1934) with modifications which have been described previously (Luduena, von Euler, Tullar and Lands, 1957; Lands, Luduena, Hoppe and Oyen, 1958). In two experiments, bronchoconstriction was produced with histamine phosphate added to the Krebs-Henseleit solution (1 : 8 million, as base) in the reservoir. In the third experiment carbachol (1 : 10 million) was used instead of histamine.

The technique of Shore and Olin (1958) with the modifications described by Lund (1959) was used to estimate biochemically the concentration of catecholamines. Direct development of fluorescence in dilutions of the original solution after treatment with iodine was of questionable value because of the brown colour of the solution caused by irradiation. For this reason the phenylephrine solutions were extracted with sodium chloride-saturated butanol. The solvent phase was added to 2 volumes of heptane and then extracted with 0·01N hydrochloric acid. Aliquots of the acid phase were taken for fluorescence development by iodine oxidation. Fluorescence was developed at pH 3·0 and 5·0 to give a differential estimation of adrenaline and noradrenaline.

RESULTS

Experiment I. A solution containing phenylephrine hydrochloride (1 mg./ml.) was irradiated for 3 hr. and then tested on two lungs. With one of the preparations, a dose of 0·025 ml. (25 μg. in terms of the original concentration of phenylephrine) produced bronchodilation comparable to that of 0·5 μg. of adrenaline (as base). In the other preparation the effect of 0·025 ml. was slightly less than that of 0·5 μg. of adrenaline. A 400 μg. dose of non-irradiated phenylephrine produced much less bronchodilation than 0·5 μg. of adrenaline. In terms of adrenaline, the bronchodilator effect of the irradiated solution corresponded to a concentration of approximately 20 μg./ml. or to the conversion of about 2·2 per cent of the original content of phenylephrine.

Experiment II. Two solutions were irradiated for 3 hr. One (A) contained 10 mg. and the other (B), 5 mg. of phenylephrine hydrochloride per ml. The solutions were kept in the refrigerator and tested the following day on the perfused lung preparation. Bronchodilatation was obtained with both solutions. On this preparation, a non-irradiated phenylephrine solution of 5 mg./ml. was approximately one-fourth as active as

solution B. Tested in comparison with adrenaline, 0·005 ml. of both solutions A and B produced approximately the same bronchodilator effect; a dose of 0·2 μg. of adrenaline was slightly more active.

From this it follows that if the activity were due to adrenaline alone, both solutions would contain 40 μg./ml. of adrenaline. But if no decomposition of the phenylephrine, other than that changed to adrenaline, had occurred, the adrenaline content would be 40 μg./ml. less the activity of the unchanged phenylephrine. This was found in a non-irradiated solution of 5 mg./ml. to be 1/4 of the activity of the irradiated solution of the same strength (solution B). Therefore the phenylephrine activity of solution B would be 1/4 of its total activity, i.e. 10 μg./ml. and its adrenaline content 30 μg./ml. As the phenylephrine activity in the 5 mg./ml. irradiated solution is equivalent to 10 μg. of adrenaline, that of solution, containing 10 mg./ml. phenylephrine, contributes 20 μg./ml. activity. Thus solution A would contain 20 μg./ml. of adrenaline. Obviously some decomposition of phenylephrine has occurred as shown by the changes in activity and the discoloration and therefore the adrenaline content would be between 20 and 40 μg./ml. for solution A and between 30 and 40 μg./ml. for solution B. These values were higher than those obtained by the fluorescence tests.

TABLE I

BRONCHODILATOR ACTIVITY OF ADRENALINE PHENYLEPHRINE AND IRRADIATED PHENYLEPHRINE SOLUTIONS (GUINEA-PIG LUNG)

Drugs*	Dose μg.*	Decrease in perfusion pressure (cm. H_2O)	
		Mean ± s.e.	Range
Adrenaline bitartrate	0·5	16·1 ± 2·6	5·0–27·5
	1·0	22·4 ± 2·1	12·0–28·5
	2·0	31·2 ± 1·8	25·5–39·0
	4·0	37·6 ± 2·0	26·0–48·5
Phenylephrine hydrochloride	165·0	5·2 ± 1·7	0·0–12·5
	330·0	11·1 ± 2·5	0·0–16·5
	660·0	15·1 ± 2·9	0·0–28·5
	1320·0	16·3 ± 3·8	0·0–26·5
Irradiated †phenylephrine hydrochloride ..	82·0	16·0 ± 2·7	4·0–29·0
	165·0	22·5 ± 2·3	7·0–29·5
	330·0	25·6 ± 1·6	17·5–33·5
	660·0	33·8 ± 1·7	26·0–41·0

* In terms of the bases.
† In terms of the original concentration of phenylephrine.

Non-irradiated phenylephrine solutions did not fluoresce when subjected to the iodine test procedure. The irradiated solutions (A and B) fluoresced at both pH 3 and 5 and gave values equivalent to an adrenaline content of 12–20 μg./ml. Noradrenaline shows little fluorescence at pH 3 (Shore and Olin, 1958; Lund, 1959) indicating that the catecholamine found was probably adrenaline because the irradiated solutions showed substantial activity when examined for bronchodilator action.

Experiment III. A solution (200 ml.) of phenylephrine hydrochloride (5 mg./ml.) in distilled water was prepared and samples irradiated as in the previous experiments (50 ml./Petri dish) for 3 hr.

The original non-irradiated solution, the irradiated solution, and a solution of adrenaline were tested on 10 guinea-pig lungs. Four graded doses of each solution were tested on each lung, the 12 doses being injected in a randomised sequence. This was important since in some preparations the sensitivity to the bronchodilators increased during the testing period. The tests were made over a period of several days. During this time, the phenylephrine solutions and a stock solution of adrenaline bitartrate (1 mg./ml. as base) were kept in closed containers in the refrigerator at approximately 4°.

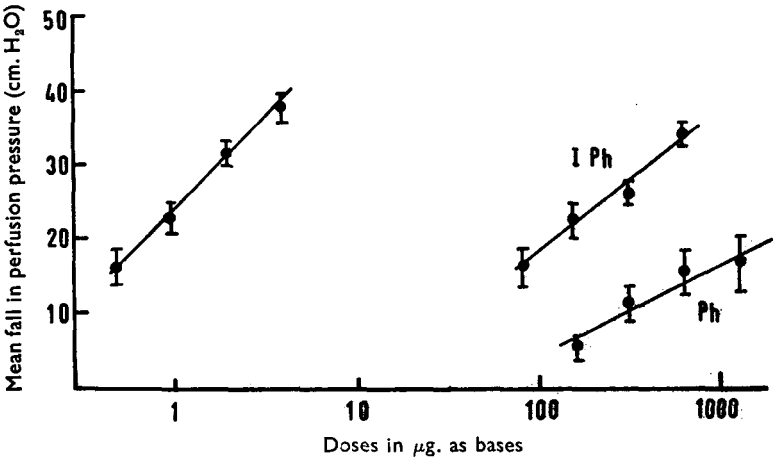

FIG. 1. Bronchodilator effect on perfused guinea-pig lung of adrenaline, phenylephrine (Ph) and irradiated phenylephrine (IPh).

At the dosage used, adrenaline and irradiated phenylephrine produced bronchodilation as measured by the fall of perfusion pressure. At the 165 and 320 μg. doses, phenylephrine produced no dilatation in 3 out of the 10 lungs, and 2 out of the 10 lungs for the respective doses. One of the preparations failed to respond to any of the 4 doses used.

The effects of the various doses on the perfusion pressure (mean ± s.e. and range) are presented in Table I. By plotting the mean response (fall of perfusion pressure) against the dose on semi-log paper approximate linear regression lines were obtained (Fig. 1).

The results show that ultra-violet irradiation produced an approximate ten-fold increase in bronchodilator activity of the phenylephrine solutions. Therefore, approximately nine-tenths of this activity is due to a compound or compounds formed during the period of irradiation. By comparing the highest and the lowest point of the dose-effect curve of irradiated phenylephrine (I.Ph) with the corresponding points in that of adrenaline, the latter (as base) was found to be 170 to 260 times more active. As each ml. of the original 5 mg./ml. solution contained phenylephrine base, 4·1 mg., the above activity ratios correspond to an adrenaline content of 24·4 to 15·4 μg./ml. This would be the amount of adrenaline if all the

phenylephrine had been destroyed but, if only a small amount had been destroyed by irradiation, that remaining would contribute approximately one-tenth of the activity of the irradiated solution. Then the content of adrenaline would be nine-tenths of the above values, i.e., 14 to 22 μg./ml. Obviously some decomposition occurred as indicated by the increase in biological activity and therefore the range of estimates of the adrenaline content should be extended to include maximal and minimal phenylephrine decomposition. This gives a range of 14 to 24·5 μg./ml. Four photofluorometric determinations of adrenaline made on different days gave a mean $\pm$ s.e. of 24·3 $\pm$ 3·8 μg./ml. This agrees, within the error of the methods, with the bioassay determination.

Discussion

The most likely explanation for the increase in bronchodilator activity of phenylephrine solutions after ultra-violet irradiation is that a small amount of adrenaline was formed. This would require the oxidation of the ring in the *p*-position. Other catecholamines such as dopamine and noradrenaline are bronchodilators, but their activity is less than that of adrenaline. The results of the fluorescence tests give adrenaline values which, considering the error of the methods, are not different from those obtained by estimating bronchodilator activity.

The investigation of Ewing and others (1931), Konzett and Weis (1938, 1939), Konzett (1941) and Holtz and Credner (1943) provided indirect evidence for the conversion of various monophenolic sympathomimetic amines into the corresponding catechol analogues. Oxedrine, tyramine, *p*- and *m*-oxyephedrine and *p*- and *m*-norsynephrine (Shepherd and West, 1952) solutions acquire higher pressor activity by irradiation. There is disagreement about the effect of ultra-violet irradiation on ephedrine solutions. According to Ewing and others (1931) irradiation reversed the pressor effect of ephedrine solutions (small doses of the catechol analogue of ephedrine lowers blood pressure, Schaumann, 1930; Tainter, 1933). However, Konzett (1941) reported that (−)- and (+)-ephedrine and (−)- and (+)-pseudoephedrine solutions retained their pressor effect even after long irradiation although discoloration occurred.

In all those investigations, increase in activity was accompanied by discoloration of the solutions, which apparently was the result of further oxidation of the catecholamine formed. This suggests that the corresponding catechol analogue is formed in solutions of mono-phenolic amines during the process of colour production by ageing.

Acknowledgements. We are indebted to Mr. J. P. McAuliff and Dr. A. Arnold for the fluorometric determinations of epinephrine.

References

Ewing, P. L., Blickensdorfer, P. and McGuigan, H. (1931). *J. Pharmacol.*, **43**, 125–129.
Holtz, P. and Credner, K. (1943). *Arch. exp. Path. Pharmak.*, **202**, 150–154.
Konzett, H. (1941). *Ibid.*, **198**, 361–368.

Konzett, H. and Weis, W. (1938). *Klin Wschr.*, **17**, 1736.
Konzett, H. and Weis, W. (1939). *Arch. exp. Path. Pharmak.*, **193**, 440–453.
Lands, A. M., Luduena, F. P., Hoppe, J. O. and Oyen, Irene (1958). *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 744–748.
Luduena, F. P., Euler, L. von, Tullar, B. F. and Lands, A. M. (1957). *Arch. int. Pharmacodyn.*, **111**, 392–400.
Lund, A. (1959). *Acta pharm. tox. Kbh.*, **5**, 231–247.
Schaumann, O. (1930). *Arch. exp. Path. Pharmak.*, **157**, 114.
Shepherd, D. M. and West, G. B. (1952). *J. Pharm. Pharmacol.*, **4**, 671–672.
Shore, P. A. and Olin, J. S. (1958). *J. Pharmacol.*, **122**, 295–300.
Sollmann, T. and Oettingen, W. F. von (1928). *Proc. Soc. exp. Biol., N.Y.*, **25**, 692–695.
Tainter, M. L. (1933). *Arch. int. Pharmacodyn.*, **46**, 192–232.
Tainter, M. L., Pedden, J. R., and James, M. (1934). *J. Pharmacol.*, **51**, 371–386.
Verda, D. J., Kneer, L. and Burge, W. E. (1931). *Ibid.*, **42**, 383–386.
West, G. B. and Whittet, T. D. (1960). *J. Pharm. Pharmacol.*, **12**, 113*T*–115*T*.