RESEARCH PAPERS

A COMPARISON OF THE BRONCHODILATOR ACTIVITIES OF ADRENALINE AND NORADRENALINE: A PROPOSED PROCEDURE FOR THE BIOLOGICAL ASSAY OF ADRENALINE SOLUTIONS CONTAINING NORADRENALINE*

BY F. C. LU AND M. G. ALLMARK

From The Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada

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IT is well known that noradrenaline possesses a weaker bronchodilator activity than adrenaline. The exact ratio of the relative activities of these two amines, however, has not been established. For example, using the aerosol method Luduena, Ananenko, Siegmund and Miller[1] found the ratio to be 1:3 while Chen, Portman, Russell and Ensor[2], found it to be 1:60. On the isolated perfused lung of the guinea-pig Cameron and Tainter[3] and Lands, Luduena, Grant and Ananenko[4], found the ratio to be approximately 1:7 ,while Luduena *et al*[1] found the value to be 1:17. On the isolated tracheal chain of the guinea-pig McDougal and West[5] found the ratio to be 1:5. While such discrepancies might be due to differences in experimental conditions, it is of interest to note that the results obtained in 4 experiments performed by Hawkins and Schild[6] on human bronchial chains were also markedly different. Since these 4 experiments were presumably carried out under identical conditions, the existence of certain inherent differences in the response of the bronchial muscle to these two amines in different animals seems apparent. However, this was not determinable on account of the experimental design used. An attempt was therefore made to find out (1) whether the bronchodilator activities of adrenaline and similar agents could be studied by a more exact method and (2) whether significant differences in the comparative activities of adrenaline and noradrenaline could be demonstrated in different animals.

It is also known that adrenaline solutions obtained from natural sources not infrequently contain 10 to 20 per cent. of noradrenaline[7,8]. Such solutions will be less effective as bronchodilator agents than solutions of pure adrenaline although equally potent in vasopressor activity, if such solutions have been assayed only for their pressor effect; noradrenaline has been shown to have a vasopressor activity equal to or greater than that of adrenaline[9]. In order to ascertain, therefore, that an adrenaline solution will have a bronchodilator and a vasopressor activity equivalent to those of a standard solution of pure adrenaline it would seem desirable to assay that solution not only for its vasopressor but also for its bronchodilator activity. Further experiments were therefore performed to determine the feasibility of the method described herein for assaying the

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bronchodilator activity of adrenaline solutions containing 10 to 20 per cent. of noradrenaline; methods suitable for assaying the vasopressor activity have been worked out and adopted by a number of pharmacopœias.

METHOD

For these experiments the tracheal chain of the guinea-pig prepared according to the method of Castillo and de Beer[10] was employed. The contraction and relaxation of the circular muscle of the tracheal preparation were recorded on a kymograph by means of a light lever. Either Locke's or van Dyke-Hasting's solution was used in the tissue bath. After the chain had been suspended in the bath for ½ to 1 hour, the relaxant actions of *l*-adrenaline and *l*-noradrenaline or mixtures of these two amines were compared, either under normal tension or following the addition of one of the following spasmogens: acetylcholine chloride, histamine acid phosphate or barium chloride. When a spasmogen was used, it was added to the bath and allowed to act for 5 minutes. The relaxant agent was then added and allowed to act also for 5 minutes before the bath was washed out. When the sympathomimetic amines were tested under normal tension, they were added to the bath, without any other drug, and allowed to act for 5 minutes. A suitable amount of acetylcholine was then added to the bath to hasten the recovery of the tension of the tracheal muscle. After the acetylcholine had remained in the bath for 5 minutes, the bath was washed out. A recovery period of 10 minutes was found to be adequate for both types of experiments. Thus, each dose required about 20 minutes. A total of about 18 doses was tested in each experiment.

The assay was usually begun after 2 orientating doses. For most experiments the design described by Noel[11] for the assay of adrenaline was used. Following this design 4 sets of tests were carried out in each experiment. Each set consisted of 4 tests, one on each of the 2 dose levels of the standard and the unknown, given in a randomised order.

RESULTS

A comparison of the bronchodilator activity of adrenaline and noradrenaline

Table I shows the results obtained from experiments in which Locke's solution was used as the nutrient fluid. The first column indicates the spasmogens used in the various groups of experiments. In the experiments of the last group the sympathomimetic amines were tested on tracheal chains under normal tension. The bronchodilator activity of noradrenaline in terms of adrenaline in per cent. is shown in the second column. It may be noted that it varied from 4·84 to 13·52 per cent. and the geometric mean potency of the whole series was 8·54 per cent. The weighted mean and its corresponding confidence limits for each group of experiments are also listed in the table. It was found that there were significant differences in potency within each group of assays, although the differences in the weighted means of the different groups were not significant at $P = 0{\cdot}05$. Table II shows the results obtained from similar experiments in which van Dyke-Hasting's solution was used instead of

Locke's solution. The geometric mean bronchodilator activity of noradrenaline in terms of adrenaline was 6·27 per cent. This value was found to be significantly different from that of the estimates obtained from experiments performed in Locke's solution. Moreover unlike those experiments significant differences in the relative activity were found not only within the different groups but also between the weighted mean of the barium chloride group and the means of the other groups.

TABLE I

THE BRONCHODILATOR ACTIVITY OF NORADRENALINE IN TERMS OF ADRENALINE.

(1) LOCKE'S SOLUTION

Spasmogen	Potency per cent.	Standard error per cent.	Test for parallelism*
1. Acetylcholine	11·58	0·38	2·20
	11·87	0·67	0·27
	6·76	0·28	0·63
	13·69	0·46	0·10
	7·00	0·36	0·17
	10·32 (7·73–13·78)†	0·43‡	
2. Histamine	10·24	3·39	0·17
	11·19	0·65	2·18
	5·68	0·41	0·06
	8·57 (5·21–13·03)	1·48	
3. Barium..	7·57	0·47	0·73
	6·97	0·48	0·10
	4·84	0·42	0·09
	6·68 (5·24–8·52)	0·46	
4. None	13·52	2·39	0·09
	7·82	0·53	4·09
	7·09	0·81	0·40
	8·07 (6·22–10·48)	1·24	

* Test for parallelism of dose-response lines. The critical value (at P = 0·05) is 2·26 for the experiments listed in this and the following tables.
† Weighted mean and confidence limits (P = 0·05).
‡ Mean of standard errors.

It was also observed that in the experiments in which barium chloride was used as the spasmogen and van Dyke-Hasting's solution as the nutrient fluid the average standard error was the smallest. This would indicate that these assays were somewhat more precise than those carried out under other conditions. Furthermore, in these assays, the bronchodilator activity of noradrenaline in terms of adrenaline was the weakest. This would indicate that a better differentiation of these two amines would be observed under these conditions. For these reasons it was considered that barium chloride was the most suitable spasmogen and van Dyke-Hasting's solution was superior to Locke's for this type of experiment. All the following experiments were therefore conducted under these conditions.

A determination of the accuracy of the method.

In view of the fact that reproducible results were not obtained when the activities of 2 different substances were compared even in experiments carried out under identical conditions (as shown in Tables I and II), it

TABLE II

THE BRONCHODILATOR ACTIVITY OF NORADRENALINE IN TERMS OF ADRENALINE.

(2) VAN DYKE-HASTING'S SOLUTION

Spasmogen	Potency per cent.	Standard error per cent.	Test for parallelism
1. Acetylcholine	7·21	0·41	0·32
	7·28	0·48	1·73
	7·58	0·16	1·95
	9·48	1·13	0·55
	5·73	0·46	1·41
	7·45 (6·92–8·02)*	0·53†	
2. Histamine	8·92	0·67	1·19
	4·67	0·27	0·74
	5·32	0·33	0·09
	5·70 (3·94–8·25)	0·42	
3. Barium..	5·72	0·23	—
	5·90	0·46	2·27
	3·49	0·14	5·13
	3·30	0·37	0·88
	5·20	0·30	0·62
	4·69	0·39	0·52
	4·97 (3·77–5·69)	0·32	
4. None	8·37	0·88	1·38
	5·90	0·24	0·98
	8·64	0·88	0·76
	7·54	0·50	0·46
	6·31 (4·68–8·50)	0·63	

* Weighted mean and confidence limits (P = 0·05).
† Mean of standard errors.

was thought of interest to test solutions of various strengths of the same substance to check the accuracy of the results obtainable with this method. Table III shows the results of these tests. Adrenaline solutions were used as the standard and unknown in 5 and noradrenaline as the standard and unknown in 4 experiments as shown in the first column. In the second column are listed the strengths of the "unknown" solutions in terms of the standard. The differences between the true potency and the potency found, listed as actual errors, varied from 0·0 to 3·9 per cent. and were all smaller than the respective standard errors. The significant differences in the results obtained in the experiments as listed in Tables I and II are thus

TABLE III

RESULTS OF ASSAYS OF ADRENALINE AND NORADRENALINE SOLUTIONS OF KNOWN CONCENTRATION

Solution	True potency per cent.	Potency found per cent.	Standard error per cent.	Test for parallelism	Actual error per cent.	Corrected error per cent.
Adrenaline	100	102·4	6·21	1·25	2·4	2·4
"	100	96·1	4·16	—	3·9	3·9
"	100	100·0	3·98	—	0·0	0·0
"	79·4	77·7	6·46	1·35	1·7	2·1
"	79·4	77·9	4·65	1·31	1·5	1·9
Noradrenaline	100	99·5	3·30	0·29	0·5	0·5
"	100	102·0	6·08	1·31	2·0	2·0
"	79·4	81·7	4·08	0·11	2·3	2·9
"	79·4	78·0	4·67	1·01	1·4	1·8
					Average	1·9

the consequence of an inherent difference in the response of tracheal chains to these 2 amines in different guinea-pigs.

Assays on adrenaline solutions containing 10 *to* 20 *per cent. of noradrenaline.*

Since precise and accurate results were obtainable when solutions of the same amine were compared, it was considered likely that assays on adrenaline solutions containing only 10 or 20 per cent. of noradrenaline against pure adrenaline would also yield reproducible results. Table IV shows the results of a number of such assays. The results were found to be homogeneous in each of these two series of unknowns. Furthermore these experiments evidently show that adrenaline solutions containing 10 or 20 per cent. of noradrenaline have definitely weaker bronchodilator activities than solutions of pure adrenaline.

TABLE IV

RESULTS OF ASSAYS OF SOLUTIONS CONTAINING VARIOUS AMOUNTS OF ADRENALINE AND NORADRENALINE IN TERMS OF PURE ADRENALINE

Contents of "unknown"		Potency found per cent.	Standard error per cent.	Test for parallelism
Adrenaline per cent.	Noradrenaline per cent.			
90	10	92·9	7·66	0·50
90	10	92·4	8·96	0·68
90	10	79·7	8·09	0·58
90	10	89·3	6·54	—
90	10	88·5	4·26	—
90	10	91·3	2·06	—
80	20	79·8	7·12	—
80	20	70·9	11·25	1·14
80	20	76·5	3·30	1·58
80	20	92·9	5·24	3·82
80	20	84·3	2·39	—
80	20	93·7	9·52	—

The weighted means and confidence limits (P = 0·05) for the first 6 and the last 6 experiments are 90·4 (87·1 to 93·7 per cent.) and 81·9 per cent. (78·4 to 85·5 per cent.).

It is to be noted that, in addition to Noel's design as cited above, the design proposed by Vos[12] for the assay of ergometrine was used in some of these experiments. The latter design has been adopted by U.S. Pharmacopeia XIV for the assay of adrenaline and posterior pituitary extract and also used by the authors for the assay of coronary dilator drugs as reported recently[13]. In the experiments in which this design was used the parallelism of dose-response curves was not tested. Apart from this point, it appears from this limited amount of data that these two designs are both suitable for this type of experiment.

TABLE V

THE BRONCHODILATOR ACTIVITY OF AMINOPHYLLINE IN TERMS OF ADRENALINE

Potency per cent.	Standard error per cent.	Test for parallelism
0·056	0·012	0·09
0·042	0·003	1·02
0·082	0·011	0·12
0·059	0·006	1·01
0·057	0·003	3·48

A comparison of the bronchodilator activity of aminophylline and adrenaline.

Another series of experiments were conducted under the aforementioned experimental conditions. The results are listed in Table V. The weighted mean potency in this series of experiments was 0·054 per cent. and the confidence limits (P = 0·05) were 0·050—0·059 per cent.

Discussion

It is of interest to note that precise as well as accurate results were obtainable with the method described herein. The precision and accuracy are evidenced by the small standard errors and actual errors observed in these experiments. Moreover, it was found in a number of experiments, the results of which have not been cited here, that the regression of response on dose was linear. These, as well as the fact that the dose-response lines of the standard and the unknown were parallel in most experiments, may be taken as indications that this method is suitable for the assay of bronchodilator drugs.

In spite of the accuracy of this method, assays on the comparative bronchodilator activity of unlike substances did not always yield reproducible results, even in experiments carried out under identical conditions. The estimate obtained in a single experiment is thus not dependable. However, the weighted means of the different groups of assays reported in this paper on noradrenaline in terms of adrenaline (using different spasmogens), with the exception of that of one group, were not significantly different from each other. These weighted means are thus probably fairly reliable. It also appears from these results that relative bronchodilator activities of noradrenaline and adrenaline do not depend, to any great extent, upon the spasmogens used.

Significant differences in the relative activities of these two sympathomimetic amines, such as those found in these experiments, have also been noted in other tissues. Thus according to Euler[14] the vasopressor activity of noradrenaline was 1 to 5 times as potent as that of adrenaline in the cat. While it is not altogether clear as to the mechanism responsible for the differences in the comparative bronchodilator activities of these two amines as observed in different animals, it may be of interest to note that thyroxine has been found to potentiate the action of adrenaline but not that of noradrenaline[15,16]. The differences of the comparative activities thus could be the result of a difference in the function of the thyroid in the different animals.

Although the results obtained in these experiments have shown that significantly different responses to these two amines exist in different guinea-pigs, the extent of variation is far too small to account for the discrepancy in the results obtained with the aerosol method as cited above. Differences in experimental conditions and factors such as differences in the rate of absorption, elimination, etc. in different guinea-pigs may also be responsible.

The geometric means of the bronchodilator activity of noradrenaline in terms of adrenaline, in the absence of a spasmogen, were 9·08 per cent.

and 7·53 per cent. for experiments performed in Locke's and van Dyke-Hasting's solution respectively. The geometric mean of the four experiments performed by Hawkins and Schild[6] on human bronchial chains was 4·4 per cent. It is to be noted that the racemic noradrenaline was used in their experiments. Since the *d*-isomer has been found to have only 1/20 to 1/60 the activity of the *l*-isomer[1], their estimate would thus be approximately 8·5 per cent. when computed in terms of *l*-noradrenaline. Furthermore the mean potency of aminophylline in terms of adrenaline has been found by Hawkins and Schild[6] to be 0·053 per cent., which compares favourably to 0·054 per cent. as observed in the experiments reported in this paper. These facts show that the results obtained in the tracheal chain of the guinea-pig are in close agreement with those obtained in the human bronchial chains.

While the true potency of the "unknown" solution used in the experiments as listed in Table IV is not known, the sum of the potencies of its two individual ingredients may be taken as its theoretical potency, assuming that there is no interference in activity when the two amines are present in the same bath. Thus as the bronchodilator activity of noradrenaline in terms of adrenaline under these experimental conditions was found to be approximately 5·0 per cent. (Table II), the potencies of the two "unknown" solutions (containing 90 per cent. of adrenaline + 10 per cent. of noradrenaline and 80 per cent. of adrenaline + 20 per cent. of noradrenaline) would be approximately 90·5 per cent. (90 per cent. + 0·5 per cent.) and 81·0 per cent. (80 per cent. + 1·0 per cent.) respectively. The results obtained in these experiments (90·4 per cent. and 81·9 per cent. respectively) are thus in fairly good agreement with the theoretical values. It may be concluded therefore that following the procedure described herein the tracheal chain of guinea-pigs is suitable for the standardisation of adrenaline solutions for their bronchodilator effect, while their vasopressor effect may be assayed by the blood pressure method.

It is to be noted that while this work was in progress a paper by West[17] appeared, suggesting, for reasons similar to those stated at the beginning of the present paper, that pharmacopœial adrenaline solutions should contain not more than 10 per cent. of noradrenaline; the determination of noradrenaline content is to be made in conjunction with a determination of either (*a*) the concentration of adrenaline (as by a chemical or a chromatographic method) or (*b*) the combined concentration of adrenaline and noradrenaline (as by a blood pressure method). Such a restriction on the noradrenaline content of adrenaline solutions will undoubtedly have its merits in the standardisation of these solutions for their bronchodilator and vasopressor effects. However, it should be pointed out that, in view of the fact that a variation in these concentrations (usually to the extent of 10 per cent.) is also permissible, adrenaline solutions standardised according to these criteria may possess a much weaker bronchodilator or a much stronger vasopressor activity than is indicated by their noradrenaline content. For example, adrenaline solutions containing slightly less than 10 per cent. of noradrenaline and having a combined concentration of

adrenaline and noradrenaline nearly 10 per cent. lower than the concentration of a solution of pure adrenaline, will possess a bronchodilator effect much weaker than that of the reference standard. In other words, their bronchodilator activity will be equivalent to that of solutions containing about 20 per cent. of noradrenaline but having a combined concentration of adrenaline and noradrenaline equal to that of the standard. On the other hand, adrenaline solutions containing slightly less than 10 per cent. of noradrenaline and having a concentration of adrenaline nearly 10 per cent. higher than that of a standard will possess a vasopressor effect much stronger than that of the standard. It is evident, therefore, that in order to ascertain that adrenaline solutions will possess a bronchodilator as well as a vasopressor activity equivalent to that of a standard preparation, the determination of these two activities as proposed in this paper would be more satisfactory than the determination of noradrenaline content.

Moreover, it should also be pointed out that most chemical and chromatographic methods are not entirely satisfactory for the standardisation of commercial adrenaline solutions in which different preservatives are invariably present. For example, the chemical method described by Auerbach[18] for the determination of noradrenaline is suitable only for adrenaline preparations in the powdered form; the chemical method described by Euler and Hamberg[19] yielded rather unreliable results as shown by West[17]; the chromatographic method usually has to be supplemented with a biological method in order to obtain accurate results[17,20]. Thus technically the method proposed in the present paper would also appear to be more satisfactory.

While the data pertaining to the bronchodilator and vasopressor activities *per se*, as obtained with the tracheal chain and the blood pressure methods, are adequate for the standardisation of adrenaline solutions, the noradrenaline content of these solutions may be computed from these data when this is desired. This computation may be made readily by using the formula given by Gaddum and Lembeck[21].

Summary

1. For the study of the comparative bronchodilator activities of adrenaline, noradrenaline and aminophylline a method has been described using the tracheal chain of guinea-pigs. The method has been found to yield data suitable for proper statistical treatment. The results from assays on different solutions of the same substance have been found to be accurate as well as precise. On the other hand, the ratios of the bronchodilator activities of different substances, while being precise, vary significantly in different experiments; the possible mechanism responsible for such differences have been discussed. The mean of the estimates from a group of assays, however, appears to give a fairly reliable result.

2. The mean bronchodilator activity of noradrenaline in terms of adrenaline has been found to be from 5·0 to 10·3 per cent. in the different groups of these experiments, and that of aminophylline has been found to be 0·054 per cent.

3. The method described herein, used in conjunction with the blood pressure method, seems to be suitable for the assay of adrenaline solutions containing 10 or 20 per cent. of noradrenaline.

REFERENCES

1. Luduena, Ananenko, Siegmund and Miller, *J. Pharmacol.*, 1947, **95**, 155.
2. Chen, Portman, Russell and Ensor, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 273.
3. Cameron and Tainter, *J. Pharmacol.*, 1936, **57**, 152.
4. Lands, Luduena, Grant and Ananenko, *ibid.*, 1950, **99**, 45.
5. McDougal and West, *Brit. J. Pharmacol.*, 1953, **8**, 26.
6. Hawkins and Schild, *ibid.*, 1951, **6**, 682.
7. Goldenberg, Faber, Alston and Chargoff, *Science*, 1949, **109**, 534.
8. Auerbach and Agnell, *ibid.*, 1949, **109**, 539.
9. von Euler, *Methods in Medical Research*, Year Book Publishers, Chicago, 1951, **3**, 131.
10. Castillo and de Beer, *J. Pharmacol.*, 1947, **90**, 104.
11. Noel, *ibid.*, 1945, **84**, 278.
12. Vos, *J. Amer. pharm. Ass., Sci. Ed.*, 1943, **32**, 138.
13. Lu, Allmark, Carmichael, MacMillan and Lavallee, *J. Pharm. Pharmacol.*, 1953, **5**, 94.
14. von Euler, *Pharmacol. Rev.*, 1951, **3**, 247.
15. Kroneberg and Huter, *Klin, Wschr.*, 1951, **29**, 649.
16. Kroneberg, *Arch. exp. Path. Pharmak.*, 1952, **216**, 240.
17. West, *J. Pharm. Pharmacol.*, 1952, **4**, 95.
18. Auerbach, *Drug Standards*, 1952, **20**, 165.
19. von Euler and Hamberg, *Science*, 1949, **110**, 561.
20. Crawford and Outschoorn, *Brit. J. Pharmacol.*, 1951, **6**, 8.
21. Gaddum and Lembeck, *ibid.*, 1949, **4**, 401.