RESEARCH PAPERS

THE ASSAY OF SOME CORONARY DILATOR DRUGS IN ISOLATED MAMMALIAN HEARTS AND DOG HEARTS *IN SITU**

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Received October 6, 1952

ALTHOUGH cortunon, a proprietary aqueous extract of mammalian liver, has been demonstrated experimentally to have a coronary dilator action,[1] clinically both favourable and unfavourable results have been observed.[2] In view of the fact that this drug is not a pure chemical and has not been biologically standardised, it was thought that the discrepancy observed clinically might be due, at least partly, to a lack of uniformity of potency among different batches. The present study was initiated to find a method suitable for the assay of such drugs and to determine the variation in potency of different batches of cortunon.

With methods involving less elaborate statistical computations than the one to be described, Anrep, Barsoum, Kenaway and Misrahy[3] have found that khellin has at least 4 times the activity of aminophylline on the coronary musculature and Henderson, Shipley and Chen[4] have found that paveril, a proprietary synthetic analogue of papaverine, caused coronary dilatation of a degree and duration equal to or greater than papaverine. In order to verify their results these drugs were also studied using the method described herein.

METHOD

Experimental procedure. In all experiments the coronary inflow was measured by means of a perfusing-recording apparatus, the details of which have been described elsewhere[5] except that the perfusion pressure was 60 instead of 100 cm. of water, based on findings given in another paper.[6]

Experiments were carried out in the isolated rabbit or dog heart or dog heart *in situ*. In the isolated rabbit heart experiments, the coronary vessels of an excised heart were perfused with oxygenated Locke's solution through a cannula inserted into the aortic stump, and in the isolated dog heart experiments defibrinated blood was used as the perfusate. In the "*in situ*" dog heart experiment the following procedure was adopted: The animal was anæsthetised with pentobarbital. The thorax was opened, under artificial respiration, by removing the anterior portion of the left fourth rib. The pericardium was slit open and fastened to the incision edges of the chest wall to raise and stabilise the

* Presented in part at the Annual Meeting of the Canadian Physiological Society, October 26, 1951, Kingston, and the Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics, April 15, 1952, New York City.

heart. The anterior descending branch of the left coronary artery was isolated for cannulation. After the animal was heparinised (1000 units/3 kg. intravenously), both the central portion of a carotid artery and the peripheral portion of the isolated coronary artery were cannulated. The carotid cannula was then connected to the reservoir end of the perfusing-recording apparatus and the coronary cannula to the outflow end. In this manner, the cannulated coronary artery was perfused with blood from the cannulated carotid artery while the rate of flow was determined when desirable.

The drugs to be tested were dissolved in distilled water except in the case of khellin which was dissolved in 30 per cent. sodium benzoate solution. The cortunon was obtained in the form of a 7·5 per cent. aqueous solution based on total solids. The proper amounts of these solutions were diluted to 1 ml. with the perfusate immediately before their injections were made, usually at 5-minute intervals, into a short piece of rubber tubing connecting the apparatus with the aortic or coronary cannula. The rate of coronary flow was recorded before and after each injection and the rate of increase in coronary flow was computed from these figures.

The Design. In order to reduce the error caused by the changes in sensitivity of the coronary vessels, a design similar to that proposed by Vos for the assay of ergometrine in the rabbit uterus[7] was used. According to this design injections of the standard at a fixed dose and of the unknown at variable doses were made alternately, permitting comparison of the dose and effect of the unknown with the dose and the mean effect of the standard immediately preceding and immediately following the unknown. The doses of the unknown were so chosen that some would produce effects greater than the respective means of the standard, and some smaller. After having completed a number of injections the doses of the standard and the unknown were increased. Such a procedure was adopted because the sensitivity of the coronary vessels usually declined significantly as the perfusion continued and because it permitted the estimation of relative potencies over a wider dosage range. Usually 3 series of injections were made in each experiment.

In respect to the reading category, it was found that the employment of the mean increases in coronary flow usually yielded results more precise than the employment of maximum increases. The mean increase in coronary flow was read over the same length of period for both the standard and the unknown in a series of injections. The length of period for each series of injections corresponded approximately with the average duration of the effect of the standard in that particular series. This procedure was followed for all experiments unless otherwise stated. The protocol of a typical experiment is shown in Table I.

The calculations. Table II shows the analysis of the data in Table I. The doses, as well as the responses, were converted into logarithms. The successive logarithmic responses of the standard were interpolated and then compared with the corresponding value of the unknown. Table III shows the calculations, using the X and Y values listed in Table

II, the principle of computation having been outlined by Vos.[7] In this case the potency found was 77·9 per cent. and the standard error was 5·1 per cent. while the true potency was 80 per cent. The logarithmic instead of arithmetic responses were used in the calculations, because it was found that the log dose and log response had a linear relation and the employment of arithmetic responses for the computation yielded potency estimates slightly less precise. For example, in this assay such calculations gave a potency of 82·8 per cent. and a standard error of 6·9 per cent. as compared to 77·9 per cent. and 5·1 per cent. respectively.

TABLE I

PROTOCOL OF A TYPICAL ASSAY

Dose Number	Dose in ml.		Coronary flow in ml./minute		
	Standard	Unknown	Control	After injection	Increase
1	0·1	—	22·80	31·14	8·34
2	—	0·1	19·18	23·54	4·36
3	0·1	—	17·59	23·26	5·67
4	—	0·2	17·43	31·31	13·88
5	0·1	—	19·00	27·53	8·53
6	—	0·075	18·75	23·08	4·33
7	0·1	—	18·69	28·09	9·40
8	0·2	—	19·79	31·31	11·52
9	—	0·2	22·00	29·39	7·39
10	0·2	—	22·34	30·32	7·98
11	—	0·15	22·52	27·40	4·88
12	0·2	—	21·28	27·68	6·40
13	—	0·4	25·33	33·14	7·81
14	0·2	—	26·15	32·02	5·87
15	0·4	—	23·36	30·32	6·96
16	—	0·4	23·75	30·00	6·25
17	0·4	—	21·92	28·94	7·02
18	—	0·6	22·27	28·94	6·67
19	0·4	—	21·12	25·90	4·78
20	—	0·3	21·59	25·00	3·41
21	0·4	—	20·36	26·76	6·40

TABLE II

ANALYSIS OF DATA IN TABLE I

Dose number	Log-dose × 100		Log-increase in coronary flow			X	Y
			Standard		Unknown		
	Standard X_s	Unknown X_u	Observed	Interpolated, Y_s	Y_u	(X_s-Y_u)	(Y_s-Y_u)
1	1·0000	—	0·9212	—	—	—	—
2	—	1·0000	—	0·8374	0·6395	0·0000	0·1979
3	1·0000	—	0·7536	—	—	—	—
4	—	1·3010	—	0·8434	1·1424	−0·3010	−0·3001
5	1·0000	—	0·9310	—	—	—	—
6	—	0·8751	—	0·9421	0·6365	0·1249	0·3156
7	1·0000	—	0·9731	—	—	—	—
8	1·3010	—	1·0614	—	—	—	—
9	—	1·3010	—	0·9817	0·8686	0·0000	0·1131
10	1·3010	—	0·9020	—	—	—	—
11	—	1·1761	—	0·8541	0·6884	0·1249	0·1657
12	1·3010	—	0·8062	—	—	—	—
13	—	1·6021	—	0·7874	0·8927	−0·3011	−0·1053
14	1·3010	—	0·7686	—	—	—	—
15	1·6021	—	0·8426	—	—	—	—
16	—	1·6021	—	0·8445	0·7959	0·0000	0·0486
17	1·6021	—	0·8463	—	—	—	—
18	—	1·7782	—	0·7629	0·8241	−0·1761	−0·0612
19	1·6021	—	0·6974	—	—	—	—
20	—	1·4771	—	0·7428	0·5328	0·1250	0·2100
21	1·6021	—	0·8062	—	—	—	—

TABLE III

FURTHER ANALYSIS OF DATA IN TABLE I

Parameter	Formula	Value
N	Number of doses of unknown	9
S(X)	Sum of X	−0·4034
$S(X^2)$	Sum of X^2	0·2591
S(Y)	Sum of Y	0·5843
$S(Y^2)$	Sum of Y^2	0·3304
S(XY)	Sum of XY	0·2192
x	X(X)/N	−0·0448
y	S(Y)/N	0·0649
(x^2)	$S(X^2)-x\ S(X)$	0·2410
(y^2)	$S(Y^2)-y\ S(Y)$	0·2925
(xy)	$S(XY)-x\ S(Y)$	0·2454
b	$(xy)/(x^2)$	1·0183
M	$x-y/b$	1·8915
Potency in %	100 (Antilog M)	77·90%
Reduced (y^2)	$(y^2)-b(xy)$	0·0426
s^2	Reduced $y^2/(N-2)$	0·0061
V(X)	$\frac{s^2}{b^2}\left(\frac{y^2}{b(xy)}+\frac{1}{N}\right)$	0·0008
sM	$\sqrt{V(X)}$	0·0282
Standard error	2·303 sM (100) (Antilog)M	5·06%

In order to demonstrate the precision obtainable with this method, a number of assays were performed on solutions of several drugs of various strengths. Table IV summarises the results. Both the true potencies and the potencies found for these solutions are listed. The actual errors and percentage errors were computed from these figures. The average percentage error was 2·5. It will be noted that the values obtained by dividing the standard errors into the respective actual errors, as indicated by t, are between 0·00 and 0·57. These values are all smaller than the t values expected at P = 0·05, indicating that the standard errors provided a valid measure of the reliability of the individual assays.

For each of the cortunon assays and the assays of the comparative activities of aminophylline and khellin, and papaverine and paveril, two or more experiments were performed. The weighted mean potency and the confidence limits for these estimates were calculated according to the usual method.[8]

TABLE IV

RESULTS OF ASSAYS OF "UNKNOWN" SOLUTIONS

Drug	No. of doses of 'unknown'	True potency per cent.	Potency found per cent.	error Standard	error Actual	Percentage error	t
Cortunon	6	100	104·1	10·2	4·1	4·1	0·40
"	7	100	100·0	4·6	0·0	0·0	0·00
"	6	125	122·4	8·9	2·6	2·1	0·29
"	9	75	69·4	9·6	5·6	7·5	0·57
"	9	80	77·9	5·1	2·1	2·6	0·41
"	9	50	50·4	4·5	0·4	0·8	0·09
Aminophylline	10	80	83·2	9·4	3·2	4·0	0·34
"	8	80	77·5	8·9	2·5	3·1	0·28
Papaverine	9	100	100·6	32·6	0·6	0·6	0·02
"	6	100	102·7	7·9	2·7	2·7	0·34
"	8	50	49·8	6·4	0·2	0·4	0·03
					Average	2·5	

RESULTS

Cortunon. In Table V are listed the relative potencies of 10 different batches of cortunon. Batch 91–1 was used as the standard of reference for all the assays except the last 4 in which the test solutions were compared with batch 91–2. It can be seen that there are significant differences in potency among these batches. Batch 91–2 has the highest potency and 98–3 the lowest. The comparative potencies of these two batches were also

TABLE V

RESULTS OF ASSAYS OF DIFFERENT LOTS OF CORTUNON

Batch number	Test object	Weighted mean potency per cent.	Confidence limits (P = 0·05) per cent.
12—1	Isolated rabbit heart ..	97·6	82·3 — 115·8
12—2	,, ,, ,, ..	84·6	69·7 — 102·7
26—1	,, ,, ,, ..	78·7	67·0 — 92·5
26—2	,, ,, ,, ..	87·7	77·2 — 99·7
31—1	,, ,, ,, ..	79·5	67·6 — 93·5
31—2	,, ,, ,, ..	90·8	76·1 — 108·3
91—2	,, ,, ,, ..	111·4	92·5 — 134·2
0106*	,, ,, ,, ..	67·4	53·5 — 84·9
98—3*	,, ,, ,, ..	32·0	25·5 — 40·1
98—3*	Isolated dog heart ..	31·3	21·1 — 67·5
98—3*	*In situ* dog heart ..	36·4	32·4 — 40·8

* Batch 91–1 was used as the standard for all the assays except the last four in which the test solutions were compared with lot 91–2.

tested on the isolated as well as the intact dog heart. The differences among the values obtained by means of these different methods are non-significant as revealed by a χ^2 test.

Aminophylline and Khellin. The coronary dilator action of khellin was compared with that of aminophylline both on the isolated rabbit heart and on the intact dog. The results are listed in Table VI. It may

TABLE VI

COMPARISON OF CORONARY DILATOR ACTIVITY OF KHELLIN IN TERMS OF AMINOPHYLLINE

Test object	Weighted mean potency per cent.	Confidence limits (p = 0·05) per cent.
Isolated rabbit heart	857·2	764·6 — 960·9
In situ dog heart	331·1	198·0 — 553·3

The doses of aminophylline were 1·0 to 4·0 mg. in the isolated rabbit heart experiments and 2·0 to 8·0 mg. in the *in situ* dog heart experiments. The corresponding doses for khellin were 0·1 to 1·0 mg. and 0·5 to 5·0 mg.

be seen that khellin is about $8\frac{1}{2}$ times more potent than aminophylline on the isolated rabbit heart and only a little over 3 times more potent in the *in situ* dog heart. A χ^2 test showed that these values are significantly different.

Papaverine and Paveril. It was noticed that paveril, in the isolated rabbit heart, exerted a much shorter action than papaverine. We therefore calculated the comparative activities using both the maximum and the mean responses. Thus paveril was found to have only 15·9 per

cent. of the activity of papaverine when the mean responses were compared while their maximum activities were quite similar. Such differences in the duration of action were not observed in the dog heart experiment. Table VII summarises the results. While the values obtained in the

TABLE VII

COMPARISON OF CORONARY DILATOR ACTIVITY OF PAVERIL IN TERMS OF PAPAVERINE

Test object	Reading category	Weighted mean potency per cent.	Confidence limits (P = 0·05) per cent.
Isolated rabbit heart ..	Mean ..	15·9	14·5 — 17·4
,, ,, ,, ..	Maximum..	96·9	79·7 — 117·7
Isolated dog heart ..	Mean ..	35·7	30·6 — 41·6
,, ,, ,, ..	Maximum..	37·3	32·4 — 42·9
In situ dog heart ..	Mean ..	43·0	38·0 — 48·6
,, ,, ,, ..	Maximum..	44·8	32·3 — 62·1

The doses of papaverine were 0·02 to 0·08 mg. in the isolated rabbit heart experiments, 0·05 to 0·2 mg. in the isolated dog heart experiments, and 0·2 to 0·8 mg. in the *in situ* dog heart experiments. The corresponding doses for paveril were 0·015 to 0·8 mg., 0·15 to 0·6 mg., and 0·5 to 4·0 mg.

isolated dog heart are not significantly different from those obtained in the *in situ* dog heart, both of these are significantly different from those obtained in the isolated rabbit heart, as shown by χ^2 tests.

DISCUSSION

For the evaluation of coronary dilator drugs a high degree of precision is usually not achieved with the use of a limited number of animals. This is due mainly to the significant changes in sensitivity of the coronary vessels during the course of an experiment and the great variability of sensitivity among different hearts. These obstacles were partly overcome in the present experiments by adopting the Vos[7] design. In this design a fairly large number of injections was made repeatedly in the same heart with the standard and the unknown given alternately.

Using this experimental design Vos[7] reported percentage errors from 0·0 to 9·0 with an average of 3·8. Thompson reported percentage errors from 0·4 to 8·0 with an average of 3·2, and from 0·0 to 8·6 with an average of 2·9 for the assays of posterior pituitary extract[9] and adrenaline[10] respectively. The percentage errors of our experiments (from 0·0 to 7·5 with an average of 2·5) thus compare favourably with those of the previous workers.

Although theoretically the precision of an experiment can be increased by increasing the number of injections, it was found that, in practice, a limited number of injections could be made in one experiment. Injections made when the heart was in poor condition usually gave erratic responses and augmented the errors of the assay. The number of injections made in our experiments was, as a rule, between 14 and 21, depending upon the condition of the heart. The *in situ* dog heart usually could tolerate fewer injections as compared to the isolated perfused heart. However, it is to be noted that while the number of injections in an

experiment is limited by the condition of the heart the degree of precision of an assay may be increased by repeating the experiments.

In the case of cortunon, since the differences in potency between batches 91–2 and 98–3 were quite similar as tested under different experimental conditions, it is considered likely that such differences would also exist in human cases. However, whether or not the differences in effect observed clinically[2] can be explained on the aforementioned findings remain to be studied.

In respect to the comparative activities of aminophylline and khellin, our results appear to be in fairly good agreement with those of Anrep *et al.*[3]

The action of paveril, as compared to that of papaverine, is apparently less prolonged though equally strong on the isolated rabbit heart, and weaker though equally prolonged on the dog heart. This is not in keeping with the results of Henderson *et al.*[4] The cause of the discrepancy is not yet clear. However, it may be worth emphasising that changes in sensitivity of coronary vessels during the course of an experiment do occur and that there are variations in sensitivity among different hearts. Both of these factors may alter the results significantly if precautions to overcome them are not attempted. That the paveril action is not so prolonged as that of papaverine in the isolated rabbit heart might be a result of a difference in the rates of "washing out" by the perfusing Locke's solution.

Summary

1. Methods suitable for the study of the comparative coronary dilator action of a number of drugs in the isolated perfused mammalian hearts and dog heart *in situ* are described. The methods permit estimations of relative potency as well as the reliability of the estimates.

2. Using the methods described herein, a number of drugs were studied It has been found that significant differences in potency exists among different batches of cortunon. Khellin has been found to be about $8\frac{1}{2}$ times as potent as aminophylline on the isolated rabbit heart and slightly over 3 times on the *in situ* dog heart. Paveril has been found to be equally potent though less long-lasting than papaverine in the isolated rabbit heart and equally long-lasting though less potent in the isolated dog heart and dog heart *in situ.*

References

1. Lu, *Rev. Canad. Biol.*, 1950, **9**, 219.
2. Saulnier, *Personal Communication.*
3. Anrep, Barsoum, Kenaway and Misrahy, *Brit. Heart J.*, 1946, **8**, 171.
4. Henderson, Shipley and Chen., *J. Amer pharm. Ass., Sci. Ed.*, 1951, **40**, 207.
5. Lu and Melville, *J. Pharmacol*, 1950, **99**, 277.
6. Lu, *Rev. Canad. Biol.*, 1951, **10**, 42.
7. Vos, *J. Amer. pharm. Ass. Sci. Ed.*, 1943, **32**, 138.
8. Bliss, *Vitamin Methods, Vol. II.*, Academic Press, New York, 1951, p. 445.
9. Thompson, *J. Pharmacol.*, 1944, **80**, 373.
10. Thompson, *J. Amer. pharm. Ass. Sci. Ed.*, 1945, **34**, 265.