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INACTIVATION OF ADRENALINE AND NORADRENALINE BY HUMAN AND OTHER MAMMALIAN LIVER *IN VITRO*

BY

W. A. BAIN AND JEAN E. BATTY

From the Department of Pharmacology, University of Leeds

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In a previous paper, Bain, Gaunt, and Suffolk (1937) described a biological assay method for following the course of adrenaline inactivation *in vitro*. Using this method they showed, among other things: (1) that adrenaline was only partially inactivated in cats' defibrinated blood, and that this was largely owing to the slow passage of some of the adrenaline into the red cells, whence most of it could be recovered by laking; (2) that the addition of tissue slices, particularly of liver, to a blood-adrenaline mixture, determined a relatively rapid and complete inactivation of the adrenaline.

In some subsequent work, not published in full, Bain and Dickinson (1938) showed that the mean rate of adrenaline inactivation by liver slices, as indicated by the half-inactivation time, varied with the species: it was fastest for the guinea-pig and for man, slowest for the cat, dog, and mouse, and intermediate for the rat. The results from 16 samples of human liver were reported at that time. Three of these inactivated adrenaline so slowly as to seem in a different category from the others. As two of the subjects had suffered from arterial hypertension of non-renal origin it was suggested that delayed inactivation of the transmitter of adrenergic vasomotor nerve activity—then thought to be adrenaline—might be responsible for such forms of raised arterial blood pressure.

The importance of noradrenaline was not, of course, recognized when these observations were made. It seemed desirable, therefore, in the light of subsequent events, to compare the behaviour of noradrenaline with that of adrenaline. This has accordingly been done by repeating some of the earlier work, but in parallel experiments in which both drugs were investigated at the same time under the same conditions. Most experiments were with human liver.

A preliminary note on some of the results has already appeared (Bain and Batty, 1952).

MATERIALS AND METHODS

Drug Solutions.—A concentrated stock solution of (−)-adrenaline hydrochloride was made by dissolving a known amount of the synthetic *laevo* base (British Drug Houses, Ltd.) in the calculated amount of 0.1 N-HCl, and adjusting the final volume with distilled water so that the solution contained 10 mg. base/ml. From this a dilute stock solution, containing 1 mg. base/ml., was prepared as required. A standard adrenaline solution, containing 10 μg. base/ml., was freshly prepared for each assay from the dilute stock solution.

*Laevo*noradrenaline was at first used as the hydrochloride, and later as the bitartrate monohydrate—both kindly supplied by Dr. M. L. Tainter. A stock solution, containing 1 mg. base/ml., was prepared by dissolving the appropriate amount of the salt in distilled water (1 mg. base = 1.22 mg. hydrochloride = 1.99 mg. bitartrate). Standard solutions for the assays were prepared by dilution of this stock solution.

Stock solutions were kept in the refrigerator. During the course of an assay the standard solutions were kept cool on ice.

Blood-Liver-Amine System.—Apart from a few preliminary experiments, where the inactivation of the amines in blood alone was studied, the inactivating system consisted of defibrinated blood, liver slices, and amine.

The advantages of blood as the medium in such experiments have been pointed out before (Bain *et al.*, 1937); the chief of these is the very consistent behaviour of adrenaline in it—a consistency far surpassing that in any other medium tried which could be regarded as physiological in any real sense. Furthermore, it seems obvious that the integrity of the liver cells, and hence the behaviour of the tissue slices, are more likely to approximate to the normal conditions in blood than in any artificial medium. Cat blood was always used, since the assays were done on cats.

Guinea-pig, rat, and mouse livers were taken from newly killed animals. Several mouse livers were

needed to provide sufficient material for an experiment. Cat liver was got from the animals used to supply blood for the experiments.

Specimens of human liver were obtained from the post-mortem room within 24 hr. of death and were kept in the refrigerator if there was to be any delay in using them. Up to 3 days' storage in the refrigerator does not seem to alter appreciably the amine inactivating power of liver. On one occasion an inactivation curve was repeated on a sample of human liver after 2 days', and on another after 3 days', refrigeration. In neither did the inactivation rates before and after storage differ. This agrees with some earlier results of Bain (1937, unpublished), who, in addition, found no difference between biopsy and post-mortem specimens, and that the activity of a sample of human liver removed at biopsy was not altered after 24 hr. in the refrigerator. These points suggest that observed differences in the inactivating power of different samples of human liver are real, and are not due simply to post-mortem changes.

The liver was cut into thin slices with a razor. The slices were dried between filter paper and weighed out into 1.0 g. portions. To each of the required number of hard-glass vaccine bottles, suspended in a water bath at 37° C. and containing 4.8 ml. defibrinated blood, was added a 1.0 g. portion of liver slices. At the appropriate time—which became zero time for that particular tube—0.2 ml. of the dilute solution of adrenaline or noradrenaline was added, giving, in the fluid phase of the system, an initial concentration of 40 μg. base/ml. (This concentration, though unphysiological, is convenient for assay purposes, since only very small volumes of blood are needed for the early parts of the assay, when the sensitivity of the test animal is at its lowest; and only moderate volumes are needed later when, although most of the amine may have been destroyed, the sensitivity of the test animal is likely to be higher, so that small amounts can be estimated (Bain *et al.*, 1937).) The amount of amine remaining was determined at intervals thereafter. The vaccine bottles were agitated mechanically throughout the experiment, except when samples were being drawn off for assay.

Method of Assay.—This was an extension, to include noradrenaline, of the method of "continuous assay" described in detail for adrenaline by Bain *et al.* (1937).

Several—usually three—different standard doses of each amine were used. These were injected in rotation, at regular intervals of about 3 min., into the jugular vein of a spinal cat, and the pressor responses recorded from the carotid artery. When the amount of adrenaline or noradrenaline in an experimental solution had to be determined, the injection of one of the standard solutions was replaced by the experimental one.

Since the concentration of adrenaline or noradrenaline in the experimental solutions is continually changing, bracketing of test and standard responses is impossible, and matching occurs only occasionally and by chance. In principle, then, the method consists in the determination of dose-response-time curves, with the frequent interpolation—or rather substitution—of test solutions. The adrenaline or noradrenaline content of any particular test injection is found by reference to the appropriate dose-response-time curve. In practice, and in the most favourable conditions, once an assay is established and is running smoothly alternate injections of standard and test solutions can be given, so that it is possible to estimate an "unknown" about every 6 min.

Part of a typical assay is illustrated in Fig. 1. The left-hand record is from an early stage of the experiment, before the assay proper has started: that on the right is from a much later stage, when the assay was well under way, the sensitivity of the animal had increased, and frequent injections of unknowns were being given.

For further details about technique the 1937 paper by Bain and his colleagues should be consulted.

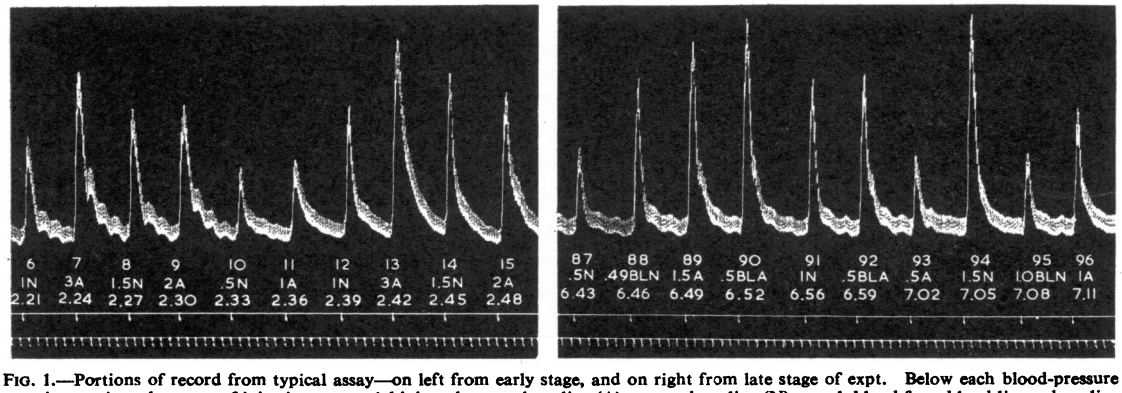

FIG. 1.—Portions of record from typical assay—on left from early stage, and on right from late stage of expt. Below each blood-pressure rise are, in order: no. of injection; material injected—μg. adrenaline (A) or noradrenaline (N), or ml. blood from blood-liver-adrenaline (BLA) or blood-liver-noradrenaline (BLN) mixture; time of administration. The signal marks indicate moment of injection, and time in ¼ min., respectively. Note how sensitivity of test animal rises in early part of expt. and has reached a steady level in later record.

RESULTS

Inactivation of Adrenaline and Noradrenaline in Blood.—Before going on to study inactivation by liver, it seemed desirable to do some preliminary experiments to see if noradrenaline added to blood alone behaved in the same way as did adrenaline. This was done in six sets of paired experiments, in each of which the inactivation of adrenaline and of noradrenaline was followed for several hours in defibrinated blood at 37° C. The initial concentration in all experiments was 40 μg./ml.

Both amines behaved similarly, the activity of the blood-noradrenaline mixtures diminishing slowly till they reached, after 2–3 hr., steady values ("equilibrium value") beyond which no further inactivation took place. The average equilibrium value for noradrenaline was 14.0 μg./ml. and for adrenaline 16.3 μg./ml.

This equilibrium phenomenon, apparently first noticed by Sugawara (1928–9), has already been investigated in some detail by Bain *et al.* (1937) for adrenaline. The interest of the present experiments is simply in the demonstration that noradrenaline behaves in the same way.

Bain and his colleagues also showed, however, that the adrenaline activity given by the equilibrium value did not represent all the adrenaline in the blood: a further amount was held inactive in association with the red cells, whence it could be released by laking. To determine if this also happened with noradrenaline, equilibrium mixtures were laked with distilled water and the amine activity again determined.

In three such experiments the average equilibrium concentration of noradrenaline was 14.7 μg./ml., or 36.7% of the initial concentration. A further 22.4% of the original amount was released on laking, giving a total noradrenaline activity representing 60% of the original amount. This is less than the 80% recovery of adrenaline reported in the earlier paper. It was matched, however, by a 57% adrenaline recovery here (probably the laking was inefficient) and so can be taken to indicate that in this further respect—the taking up of the amine by the red cells—noradrenaline behaves like adrenaline.

Inactivation of Adrenaline and Noradrenaline by Liver Slices in Blood.—When adrenaline or noradrenaline is added to blood containing liver slices inactivation of the amine proceeds in an exponential fashion, so that there is a linear relationship between the logarithm of the concentration and time. By constructing such a linear graph by eye, from the assay data for each experiment, the time for destruction of half the adrenaline or noradrenaline is readily determined. The result of one such experiment (No. 65) is illustrated in Fig. 2.

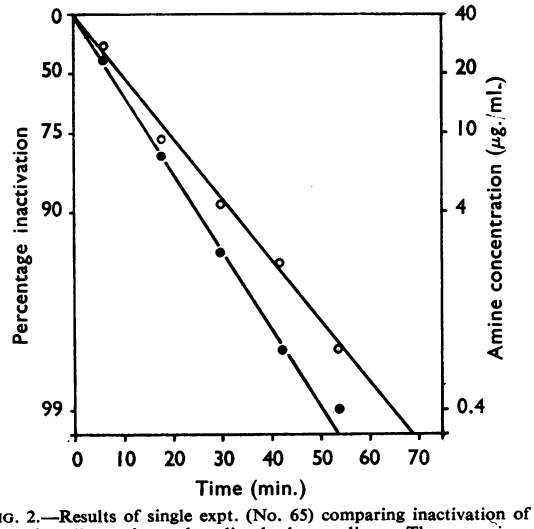

FIG. 2.—Results of single expt. (No. 65) comparing inactivation of adrenaline and noradrenaline by human liver. The regressions were drawn by eye through the experimental points. Ordinates, inactivation (%), and amine concentration (μg./ml.). Abscissa, time in min. Open circles, adrenaline; closed circles, noradrenaline. The speed of inactivation of both amines was exceptionally fast in this expt.

The average half-inactivation times, obtained in this way for different mammalian species, are summarized in Table I. It is clear that noradrenaline is destroyed more rapidly than adrenaline by the liver of each species.

TABLE I

AVERAGE HALF-INACTIVATION TIMES (MIN.) OF ADRENALINE AND NORADRENALINE WHEN INCUBATED WITH LIVER OF VARIOUS SPECIES IN BLOOD AT 37° C.

Numerals in parentheses indicate the lowest and highest values obtained.

Species	No. of Expts.	Adrenaline	Noradrenaline
Guinea-pig ..	7	15·1 (11·0–22·0)	11·2 (9·0–15·0)
Rat	9	25·3 (18·5–46·0)	19·2 (12·0–28·0)
Cat	7	45·1 (25·0–72·0)	27·6 (12·0–50·0)
Mouse	2	50·5 (50 0–51·0)	36·5 (30·0–43·0)
Man	28	16·5 (9·5–27·0)	13·1 (7·5–24·0)

Furthermore, the results with adrenaline are in good agreement with those previously published (Bain and Dickinson, 1938). The half-inactivation

time of 13 min. for human liver given in that paper was, however, derived from the 13 liver samples presumed to be "normal." When the values for the slow livers are included the average becomes 15.5 min., with a range of from 10 to 27 min. The present results are in striking accord with these.

Course of Adrenaline and Noradrenaline Inactivation.—The relatively large number of experiments with human liver made it possible to analyse these results in more detail. Before this was done, however, the precise relation between log concentration and time in a blood-liver-amine system was determined.

For this purpose the standard type of experiment was carried out in triplicate for both the amines, with human liver as the inactivator. The replicate experiments with adrenaline were designated A_1, A_2, and A_3, and those with noradrenaline N_1, N_2, and N_3. The amine was added to the appropriate tube at a planned time, and samples were taken for assay from each tube 6, 18, 30, 42, and 54 min. later. The estimated amine contents at these times in the six experiments are shown in Table II.

TABLE II

ADRENALINE AND NORADRENALINE CONCENTRATIONS (μG./ML.) ESTIMATED BIOLOGICALLY IN REPLICATE INACTIVATION SYSTEMS AT DIFFERENT TIMES (MIN.) AFTER ADDITION OF THE AMINES

Expt.	Time from Addition of Amine (min.)				
	6	18	30	42	54
A_1	30·0	8·9	4·1	1·8	0·8
A_2	28·6	8·0	—	2·6	0·6
A_3	28·5	10·8	4·7	2·2	1·0
N_1	25·7	9·0	3·1	1·1	0·4
N_2	21·0	6·4	—	0·4	—
N_3	24·0	7·3	2·9	0·9	0·3

In analysing the results the methods described by Finney (1950) were used. The data for adrenaline and noradrenaline were treated independently to determine the relation of log concentration and time, but were combined in order to compare the inactivation of the two amines. The concentrations in Table II were converted to their natural logarithms. The missing values were estimated and the degrees of freedom adjusted accordingly.

The analyses of variance for the replicate experiments with adrenaline and with noradrenaline are shown in Tables III and IV respectively. It is clear from these analyses that there is a linear relation between log concentration and time for each amine.

Furthermore, a t test applied to determine the difference between the two regressions gave $P<0.001$. The difference in slopes of the regressions of log concentration on time is thus highly significant: under identical conditions, therefore, the inactivation of each amine progresses at a different rate.

Regression Coefficients as a Measure of Inactivation Rates.—Having established the linear relation

TABLE III

ANALYSIS OF VARIANCE OF THE DATA FROM REPLICATE EXPERIMENTS ON ADRENALINE INACTIVATION BY HUMAN LIVER

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Regressions ..	22·514	1	22·514	833·85	<0·001
Deviations from regression ..	0·191	3	0·064	2·37	0·1–0·2
Between times ..	22·705	4	5·676	210·22	<0·001
,, expts.	0·076	2	0·038	1·41	>0·2
Residue ..	0·186	7	0·027		
Total ..	22·967	13			

TABLE IV

ANALYSIS OF VARIANCE OF THE DATA FROM REPLICATE EXPERIMENTS ON NORADRENALINE INACTIVATION BY HUMAN LIVER

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Regressions ..	36·611	1	36·611	892·95	<0·001
Deviations from regression ..	0·072	3	0·024	0·59	>0·2
Between times	36·683	4	9·171	223·68	<0·001
,, expts.	0·708	2	0·354	8·63	0·02–0·03
Residue ..	0·245	6	0·041		
Total ..	37·636	12			

TABLE V

THE REGRESSION COEFFICIENTS FROM THE LOG CONCENTRATION-TIME RELATION FOR ADRENALINE AND NORADRENALINE INCUBATED WITH HUMAN LIVER

Expt. No.	b Adrenaline	b Noradrenaline
21	−0 02378	−0 02582
22	−0 04467	−0·07530
24	−0·03882	−0·05131
25	−0·03949	−0·05060
27	−0·02382	−0·03045
28	−0 04075	−0·03590
31	−0 03576	−0·04594
32	−0 06222	−0·07941
33	−0·04192	−0·04873
34	−0·04323	−0·05254
36	−0·06400	−0·07163
37 (a)	−0 04368	−0·07289
37 (b)	−0·05128	−0·07481
40	−0·07163	−0·05860
41	−0·05342	−0·05223
43	−0·06611	−0 06656
44	−0 04740	−0·06271
46	−0·03915	−0·07023
49	−0·03325	−0·04611
51	−0·06145	−0·07234
55	−0·03330	−0·05346
57	−0·02516	−0·04163
58	−0·02737	−0·03873
59	−0·02882	−0·04094
61	−0·05560	−0·06228
63 (a)	−0·05478	−0·06110
63 (b)	−0·03858	−0·05237
65	−0·07244	−0·08886
Mean ..	−0·04474	−0 05655

TABLE VI

ANALYSIS OF VARIANCE OF REGRESSION COEFFICIENTS FROM 28 EXPERIMENTS WITH HUMAN LIVER

Source of Variation	Sum of Squares	d.f.	Mean Square	Variance Ratio	P
Between drugs	0·00196	1	0·00196	39·2	<0·001
,, expts.	0·01073	27	0·00039	7·8	<0·001
Residue ..	0·00136	27	0·00005		
Total ..	0·01405	55			

between log concentration and time for both amines, it was possible to analyse the data from the experiments with human liver using the regression coefficient as a measure of the inactivation rate. Table V gives the regression coefficients from the 28 experiments mentioned earlier. Analysis of these (Table VI) shows a highly significant difference between the rates of inactivation of the two amines, noradrenaline being the more rapidly destroyed. (It is perhaps worth emphasizing, however, that it is the constancy of the difference, rather than its magnitude, that contributes so greatly to its significance.)

The mean regressions are shown graphically in Fig. 3. The calculated half-inactivation times, and their fiducial limits ($P=0.95$), are 15.5 (13.8–17.7) min. for adrenaline and 12.25 (11.1–13.7) min. for noradrenaline. The corresponding values derived by inspection of the graphs drawn by eye for each experiment are 16.5 min. for adrenaline and 13.1 min. for noradrenaline (Table I).

Mechanism of Adrenaline and Noradrenaline Inactivation by Human Liver.—An enzyme system was thought to be responsible for the amine inactivating property of liver, because boiling for a few minutes destroyed this property. Of the three enzyme systems known to inactivate adrenaline and noradrenaline, only amine oxidase (Blaschko, Richter, and Schlossmann, 1937) and cytochrome oxidase (Keilin and Hartree, 1936, 1937, 1938) were considered, for the third—catechol oxidase—is probably not important in mammals (Bhagvat and Richter, 1938). As amine oxidase is inhibited by octyl alcohol and is unaffected by cyanide, whereas cytochrome oxidase is inhibited by cyanide and is unaffected by octyl alcohol, the presence of either enzyme—or of both—can readily be determined.

Control systems, consisting of blood, liver, and amine in the usual quantities, were compared with similar systems which contained in addition either 10^{-3}M-sodium cyanide or octyl alcohol.

The results with cyanide are shown in Table VII. The addition of cyanide had no marked effect upon the rate of inactivation of either amine. Octyl alcohol, on the other hand, caused a profound slowing of inactivation: in one experiment with adrenaline the destruction at 309 min. in the inhibited system was comparable to that at 33 min.

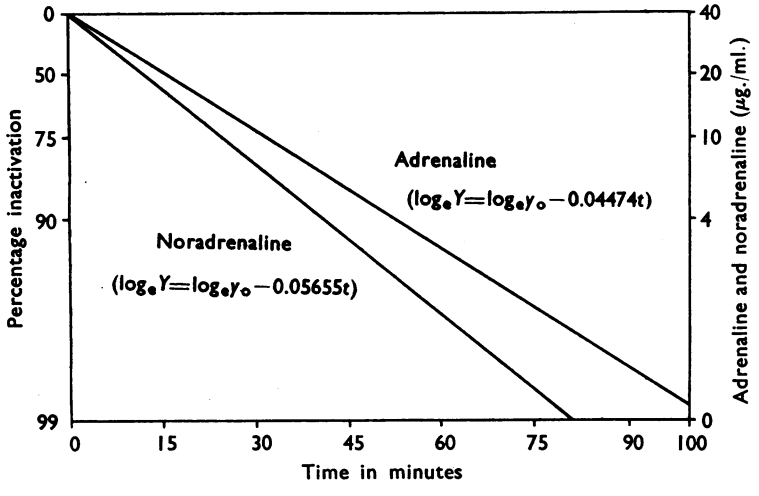

FIG. 3.—Regressions of log concn. on time for adrenaline and noradrenaline inactivation by human liver, calculated from the data of Table V. Upper line, adrenaline; lower line, noradrenaline.

TABLE VII

EFFECT OF CYANIDE ON THE HALF-INACTIVATION TIMES OF ADRENALINE AND NORADRENALINE BY THREE SAMPLES OF HUMAN LIVER

Expt.	Adrenaline		Noradrenaline	
	Without Cyanide	With Cyanide	Without Cyanide	With Cyanide
1	12·25 min.	12·5 min.	10·75 min.	8·5 min.
2	14·0 ,,	14·5 ,,	11·5 ,,	12·0 ,,
3	16·5 ,,	17·0 ,,	12·75 ,,	13·0 ,,

in the control; in the other it was equivalent at 154 min. in the presence of octyl alcohol to that at 15 min. in the control. The results with noradrenaline were similar.

It thus seems clear that the enzyme responsible for the inactivation of adrenaline and noradrenaline in these experiments was amine oxidase.

Discussion

It was hoped, in embarking on these experiments, that it would be possible either to corroborate or disprove the hypothesis (Bain and Dickinson, 1938) that delayed inactivation of the transmitter of adrenergic nerve activity might be responsible for some forms of raised arterial tension. The clinical data available about the patients were, however, quite inadequate for this purpose. Furthermore, there was no evidence that the results with human liver were not normally distributed: the earlier results of Bain and Dickinson might therefore have occurred by chance. Finally, though there is evidently a fairly wide variation in the rate of amine inactivation by different samples of liver, it is not known whether there is a corresponding variation at or near the endings of adrenergic nerves. Nor is it easy to see how the state of affairs at these endings could be determined.

In any event it is obvious that the original hypothesis, based on results with adrenaline, can now, whatever its worth, be extended to include noradrenaline.

Summary

1. When noradrenaline is added to defibrinated blood, its rate of disappearance, and its distribution between cells and serum, are similar to that of adrenaline.

2. Noradrenaline is inactivated slightly more rapidly than adrenaline when incubated in blood with liver slices of guinea-pig, rat, cat, mouse, and man.

3. There is a linear relation between log concentration and time for the inactivation of adrenaline and noradrenaline by human liver.

4. Analysis of the regression coefficients for adrenaline and noradrenaline inactivation by human liver confirms the more rapid inactivation of noradrenaline.

5. The enzyme responsible for the inactivation is amine oxidase.

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