Potency of therapeutic adrenaline in injections

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INTRODUCTION

LITTLE INFORMATION is available on the stability and hence the potency of adrenaline in injections packed locally or imported. This is important particularly in the tropics since fluctuations in temperature tend to accelerate the rate of decomposition of adrenaline in injections with a marked loss of pharmacological activity (Munch, Sloane and Latven, 1950; Backe-Hensen, Aares, Vennerod and Jensen, 1963). In view of this and the therapeutic importance of adrenaline as cardiac stimulant and bronchodilator, the present study was carried out to assess the potency of some proprietary adrenaline in injections.

SUMMARY

The potency of four samples of proprietary adrenaline in injections was compared with a reliable standard adrenaline. The potency of adrenaline was assessed by its pressor activity on the pithed rat. Of the four samples of adrenaline bioassayed, samples A and B possessed pressor activity which was significantly higher than the standard, while sample C was approximately equipotent. Sample D was the least potent compared with the standard.

Paper chromatography of samples A and D were carried out with reference to the same standard adrenaline. It was found that sample A separated out into two spots on the chromatogram; one did not correspond to the Rf value of the standard adrenaline or to the standard noradrenaline (an additional standard used in the chromatography). The extra spot, however, had a colour reaction characteristic of the standard noradrenaline. No spot could be detected on the chromatogram of sample D.

METHODS

Rats of the Sprague-Dawley strain weighing between 200 and 300 g were used. The rat was pithed by the technique described by Shipley and Tilden (1947). The blood pressure was monitored from the left common carotid artery via a Statham pressure transducer (P23AC) coupled to a Grass Polygraph (Model 5). In pithed rat preparation, adrenaline is approximately equipotent as noradrenaline (Muscholl, 1959) and the preparation could therefore be used to bioassay adrenaline.

The potency of four samples of proprietary adrenaline in injections, A, B, C and D was studied. There were four ampoules in each sample and their activity was compared with freshly prepared standard l-adrenaline bitartrate (Sigma, USA). Adrenaline was diluted in normal saline solution immediately before use. The drug was injected via the cannulated right femoral vein. The total volume injected was 0.3 ml

(0.1 ml adrenaline + 0.2 ml saline).

One dimensional paper chromatography of samples A and D were carried out using Whatman No. 1 paper. The solvent system used was n-butanol saturated with 1 M HC1 (Euler and Hamberg, 1949). The solvent was run in the descending direction at 25°C for 14 –16 hours (overnight).

The standard solutions consisted of 1-adrenaline bitartrate (Sigma, USA) and 1-noradrenaline bitartrate (Sterling-Winthrop, USA) dissolved 1 M HC1 to a concentration of 5 mg/ml.

Following chromatography, the colour reaction of catecholamines was developed with ninhydrin and potassium ferricyanide (James, 1948).

RESULTS

Bioassay of adrenaline:

The net change in systolic pressure (mmHg) of pithed rat produced by 4 samples of adrenaline in injections A, B, C and D is shown in Fig. 1. With the exception of sample D, the responses produced by the others were equal or much greater than the standard adrenaline. At a dose level of 5 ug, it was seen that (Table I) the blood pressure change due to samples A and B was significantly higher than the standard (t-test, $P < 0.001$). The activity of sample C was approximately the same as the standard, while the activity of sample D was very much lower than the standard (t-test, $P < 0.001$).

The relative potency of 4 samples of adrenaline is summarised in Table 2. Sample A was about twice the potency of the standard on its action on the blood pressure of pithed rat. Sample B was one-and-a-half times stronger than the standard, while sample C was

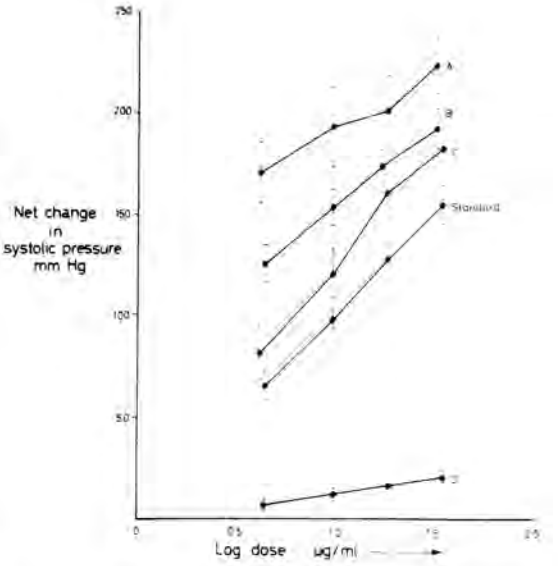

Figure 1: Net change in blood pressure of pithed rat produced by various adrenaline in injections (Samples A, B, C and D) and by standard adrenaline. Each point is the mean of 4 observations and the vertical strokes indicate the standard errors of these means.

TABLE I
CHANGE IN BLOOD PRESSURE OF PITHED RAT PRODUCED BY 5 ug OF VARIOUS ADRENALINE IN INJECTIONS

Sample	Change in blood pressure mmHg	No. of observations	P value (t-test)
Standard	97.5 ± 5.0	24	—
A	192.5 ± 20.0	4	< 0.001
B	160.0 ± 14.0	4	< 0.001
C	120.0 ± 12.5	4	—
D	12.5 ± 3.0	4	< 0.001

Figures refer to Mean ± standard error

TABLE II
RELATIVE POTENCY OF ADRENALINE IN INJECTIONS

Adrenaline in Injections	Potency Ratio = Adrenaline in Injection / Standard Adrenaline
Samples	
A	3.1 ± 1.2
B	2.2 ± 0.5
C	2.2 ± 0.5
D	1.4 ± 0.5
	0.10 ± 0.07

Figures refer to mean ± standard error of 4 (2+2) bioassay experiments.

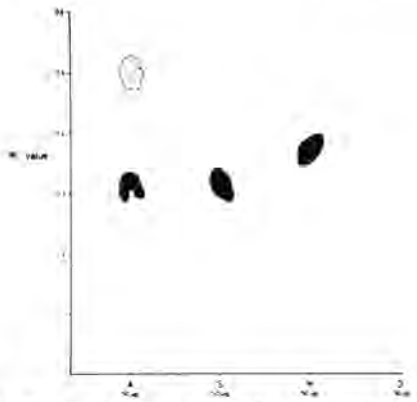

Figure 2: Paper chromatogram of adrenaline taken from Samples A and D. The standard adrenaline and noradrenaline are represented by S and N respectively.

approximately equal. With sample D, the potency was only about a tenth of the standard.

Chromatography of Samples A and D

Since the activity of adrenaline from samples A and D was very much different from the standard, it was thought of interest to investigate their purity, using one dimensional paper chromatography. The chromatogram in Fig. 2 showed that sample A separated out into 2 spots, one having a Rf value of the standard adrenaline, and the other having a Rf value which was not related to either the standard adrenaline or to the standard noradrenaline. However, the additional spot showed a colour reaction similar to that of noradrenaline.

No apparent spot corresponding to standard catecholamine was detected with sample D (fig. 2). This probably accounts for its weak effect on the blood pressure of pithed rat.

DISCUSSION

The results of the present study indicate that the potency of various adrenaline in injections varies considerably from one sample to another when compared with a reliable standard. Of the four samples of adrenaline investigated, only one of them was close to the potency of the standard, while the potency of the others was significantly different from the standard. The potency of sample A adrenaline is of particular interest, since it produced the largest change in the blood pressure of pithed rat, suggesting perhaps that the sample may be contaminated with some pressor agent. This view is supported in part by the results obtained with chromatography of the sample. Alternatively, it may be that the sample contains more than the usual amount of adrenaline of 1 mg per ml specified by British Pharmacopoeia.

On the other hand, the pressor activity of sample D was only about one-tenth of the standard. The bioassay results are consistent with those obtained with chromatography of the sample. No apparent spot could be detected on the chromatogram. Incomplete oxidation of adrenaline may contribute to the marked loss of activity. Oxidative degradation of adrenaline results in the formation of a colour complex in the solution (West, 1947) but this was visually absent in all the ampoules of the sample. According to Girard and Kirny (1950), a loss of pharmacological activity can occur with an apparently clear solution of adrenaline. Loss of activity may also arise from a chemical reaction between adrenaline and sodium metabisulphite (a recognised stabilising agent) resulting in the formation of adrenaline sulphonate. This substance is pharmacologically inactive and is invariably found to be present in adrenaline in injections (Backe-Hansen et al, 1963). Whether this substance is actually present in sample D is not known and its study requires chemical analysis which is now in progress.

ACKNOWLEDGEMENTS

Thanks are due to Mr. A. Chong, Pharmacist, University Hospital, University of Malaya, Kuala Lumpur, for the supply of the four samples of adrenaline in injections, and to Professor K. E. Chan for his interest in the investigation.

REFERENCES

1. Backe-Hansen, K., Aarnes, E.D., Vennerod, A.M., Jensen, K.B. (1963). On the stability of adrenaline in injections – a comparison of chemical and bioassay methods. *J. Pharmac.* **15**:804.
2. Euler, U.S. von, and Hamber, U. (1949), L-noradrenaline in the suprarenal medulla. *Nature, Lond.* **163**:642.
3. Girard, M.P. and Kerny, G. (1950), Adrenaline, preservation of solutions of *J. Pharma. Pharmac.* **11**:853.
4. James, W.O. (1948), Demonstration and separation of noradrenaline, adrenaline and methyladrenaline. *Nature, Lond.* **161**:851.
5. Munch, J.C., Sloane, A.B. and Latven, A.R. (1952), Adrenaline and analogues, rate of loss of potency in solution. *J. Pharma. Pharmac.* **15**:337.
6. Muscholl, E. (1959), Die konsentration von noradrenaline und adrenaline in den einzelnen abschnitten des herzens. *Arch. exp. Path. Pharmak.*, **237**:350.
7. Shipley, R.E. and Tilden, J.H. (1947), Pithed rat preparation suitable for assaying pressor substances. *Pro. Soc. exp. Biol. Med.* (N.Y.), **64**:453.
8. West, G.B. (1947), Oxidation of adrenaline in alkaline solution. *Brit. J. Pharmac. Chemo. Ther.*, **2**:121.