

THE ASSAY OF HISTAMINE, 5-HYDROXYTRYPTAMINE, ADRENALINE, AND NORADRENALINE, ON THE BLOOD PRESSURE OF THE FOWL

BY I. L. NATOFF AND MARY F. LOCKETT

From the Department of Physiology and Pharmacology, Chelsea Polytechnic, London, S.W.3

Received January 14, 1957

Histamine and 5-hydroxytryptamine, 1 $\mu\text{g./kg.}$ depress, and (–)-adrenaline and (\pm)-noradrenaline 0.5 $\mu\text{g./kg.}$ raise, the blood pressure of fowls under pentobarbitone anaesthesia. With each compound the response in mm. Hg is linearly related to log dose. The responses have been used satisfactorily for the assay of these compounds.

ACTIONS and side-actions of new drugs destined for clinical trial are usually examined in cats, dogs, and sometimes monkeys, because the physiology of these species most closely resembles that of man. However, the use of these species for biological assay and for the preliminary screening of new drugs appeared justifiable, at the present time, only if no satisfactory alternative method is known.

Highly sensitive methods exist for the assay on isolated tissues of many naturally occurring pharmacologically active substances. Nevertheless, it is often desirable to examine such compounds either in tissue extracts, or preferably after separation from other constituents of the extracts, on a variety of preparations. That the fowl might yield a suitable whole animal preparation for this purpose has therefore been examined.

METHODS

Fowls of various breeds, weighing from 1.0 to 1.6 kg., were anaesthetised by the intramuscular injection of 1.0 ml./kg. of 6 per cent (w/v) pentobarbitone sodium in a solvent composed of 10 parts (v/v) ethanol, 20 parts propylene glycol, and 70 parts water. Full surgical anaesthesia developed in 30 to 40 minutes, and could be maintained by the intramuscular injection of approximately 0.3 ml./kg. of this solution per hour. The tongue was drawn forward, and a tracheal cannula was passed through the larynx under direct vision. Gentle positive ventilation was begun at once, and was continued throughout the experiment. The fowl was placed on its side with its legs extended at right angles to its back. Feathers were plucked from over the presenting femur. An incision was made through skin parallel to, and 1 cm. behind, this bone. The skin was retracted and the muscles were separated to expose the ischiadic artery and vein. Both vessels were cannulated. Drugs were injected into the venous cannula. Mean arterial pressure was recorded from the arterial cannula by means of a Condon rat manometer¹. Heparin was used as anticoagulant.

Dose-effect curves relating graded responses of the fowl's blood pressure to drugs were determined as follows: Three or more doses of drug were selected, each of which produced a measurable but submaximal effect.

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These doses were given repeatedly in a random order; the responses obtained were measured to the nearest millimetre. The results were analysed by standard statistical methods.

Assays. Two suitable doses, which produced submaximal effects, were chosen for both standard and test solutions. The ratio of the high dose to the low dose was the same for both standard and test solutions. Letters were assigned to these doses, which were given in the order of Latin squares. Drugs were injected at regular intervals which varied from two minutes in experiments with histamine to four minutes in experiments with adrenaline. Responses were measured to the nearest millimetre.

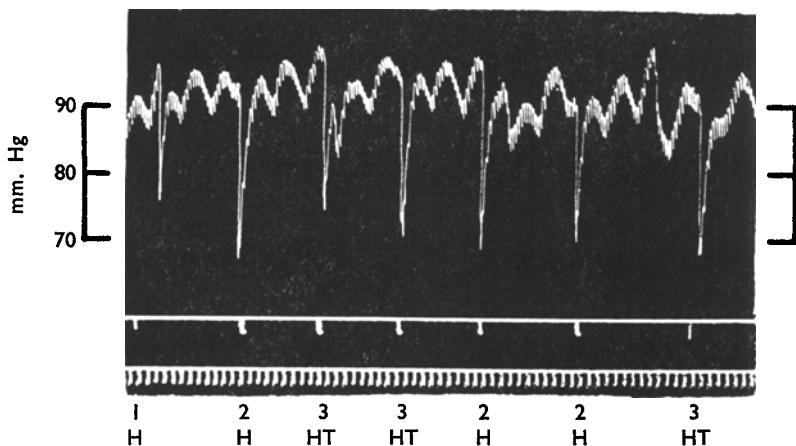


FIG. 1. The relative potency of histamine and 5-hydroxytryptamine on the fowl's blood pressure. Record of blood pressure from a fowl, wt. 0.9 kg. Histamine, H, 5-hydroxytryptamine, HT, injected i.v. Doses in μg . Time trace, 10 seconds.

Assay results were calculated by the method described by Schild², except that the following formula was used to determine the standard error of the mean: $V_m = \frac{S^2}{b^2} \left(\frac{1}{n_s} + \frac{1}{n_u} \right) + \frac{(\bar{Y}_s + \bar{Y}_u)}{b^4} \cdot V_b$, where S = root of the mean square for error, n_s and n_u are the numbers of observations made on the standard and test solutions respectively, \bar{Y}_s and \bar{Y}_u are the means of all responses to standard and to test solutions respectively, b is the slope of the log dose effect curve, and V_b is the variance of this slope.

RESULTS

Dose Effect Curves for the Action of Drugs on the Blood Pressure of the Fowl

Well ventilated fowls maintained a steady resting blood pressure, at a level between 80 and 120 mm. Hg, for many hours. The repeated injection of depressor drugs caused a small wave-like variation in the resting blood pressure which was maintained throughout the estimation of dose-effect curves, and throughout assays, of depressor drugs. These waves (Fig. 1) occurred at rates of approximately 2 to 3 a minute, and were central in origin. They did not seriously affect estimations of depressor

action. Pressor drugs did not induce variation in the resting blood pressure, and abolished that due to depressor drugs.

Histamine. The intravenous injection of histamine caused an abrupt fall of blood pressure. Submaximal depressor responses were proportional to the log dose of histamine used, and lasted for less than 1 minute. This fall was followed by a rise in blood pressure above the normal resting value before the latter was restored. The whole biphasic

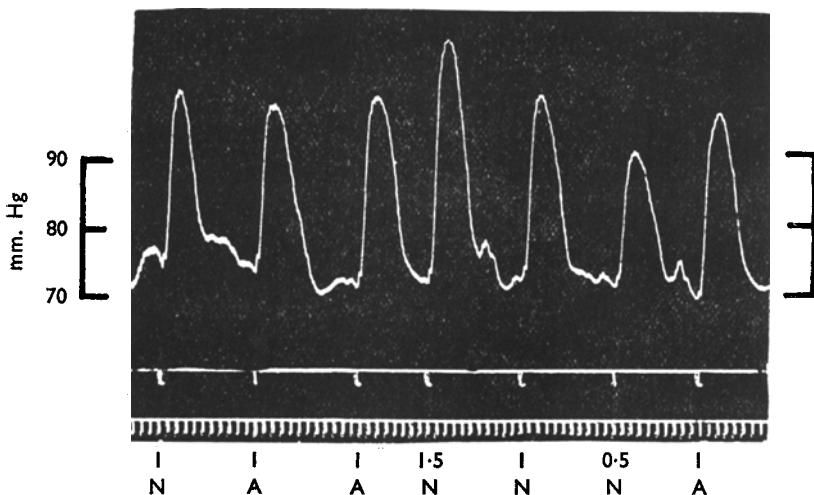


FIG. 2. The relative potency of adrenaline and noradrenaline on the fowl's blood pressure. Record of blood pressure from a fowl, wt. 1.3 kg. (—) Adrenaline, A, and (±) noradrenaline, N, injected i.v. Doses in μg . Time trace, 10 seconds.

response lasted less than 2 minutes. In thirty-two determinations of the log dose effect curve for the action of histamine on the blood pressure of the fowl, the mean weight of histamine acid phosphate required to depress the blood pressure by 20 mm. Hg. was $3.23 \mu\text{g./kg.}$, standard error 0.31. The mean slope of the curves was 38.48, standard error 2.39.

5-Hydroxytryptamine. Depressor responses to the intravenous injection of 5-HT had duration similar to, but were followed by a secondary rise in blood pressure less than that of, responses to histamine in the same bird. Tachyphylaxis occurred during the first 5 to 10 responses to 5-HT, but thereafter responses to this drug remained constant when it was injected at 3 minute intervals. When the response to 5-HT had become constant, the effect of $1 \mu\text{g.}$ histamine base equated approximately with that of $1.5 \mu\text{g.}$ 5-HT. The slopes of the dose effect curves were however a little steeper for 5-HT than for histamine in the three birds in which this comparison was made (Fig. 1).

Adrenaline and noradrenaline injected intravenously caused very similar pressor responses of the blood pressure. The effect of $1 \mu\text{g.}$ (—)adrenaline roughly equated with that of $1 \mu\text{g.}$ (±)noradrenaline in each of three birds, and the dose effect curves given by these two drugs did not differ significantly from parallel (Fig. 2).

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The Assay of Drugs on the Blood Pressure of the Fowl

Histamine. Twenty-eight assays were made in which the concentration of histamine acid phosphate in both the standard and the test solutions was known. The tracing obtained in a single 2 + 2 assay, in which each dose was given four times, is shown in Figure 3. The standard solution contained 15 $\mu\text{g./ml.}$, and the test solution 18 $\mu\text{g./ml.}$ The concentration of the test solution calculated from the data provided by this assay was 18.5 $\mu\text{g./ml.}$ The calculated slope, b , of the dose effect curve was 44.4. The value s/b was 0.14 where $s = \sqrt{\text{mean square for error.}}$

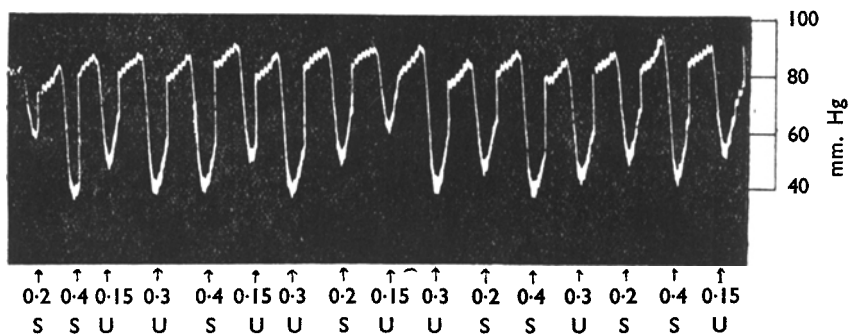


FIG. 3. Record of a 2 + 2 assay of histamine on the blood pressure of the fowl, wt. 1.6 kg. Injections of either standard, S, or test, U, solutions were made i.v. at 2 minute intervals. Doses in ml.

The Fiducial limits, $P = 0.95$, were however 13.0 to 26.5 $\mu\text{g./ml.}$ The precision of this assay could have been much increased by repetition of the randomised groups of doses, to a total of 12 or 16 trials at each dose level. A summary has been made of twenty-eight such 2 + 2 assays of histamine acid phosphate in which only four randomised groups of doses

TABLE I

SUMMARY OF THE RESULTS OF 2 + 2 ASSAYS OF HISTAMINE ON THE FOWL'S BLOOD PRESSURE

	Nos. of randomised dose groups used	
	Four	Twelve
Mean found as per cent true	103.93 \pm 2.34 (28)*	99.77 \pm 0.48 (6)*
Limits of error per cent ($P = 0.95$):		
Determined directly	-27.7 to 31.1	-14.4 to 16.9
Determined indirectly	-23.8 to 31.1	-14.1 to 15.8
Value s/b	0.01 \pm 0.08 (28)*	0.08 \pm 0.03 (6)*

* Mean \pm standard error (number of observations).

were given. This summary is found on the left side of Table I. The right side of the Table shows a similar summary of the results of six assays which differed only in that twelve randomised groups of doses were employed. It is evident that whereas assays consisting of only four groups of randomised doses can be relied upon to detect a 30 per cent difference between standard and test solutions, not less than twelve such groups are needed to ensure the detection of a 15 per cent difference between

samples. The preparation proved hardy, and gave good discrimination between doses for 5 to 7 hours: responses were evoked every two minutes.

5-Hydroxytryptamine. Three assays of 5-HT were made on the fowl's blood pressure. In each experiment 5-HT was injected at 3 minute intervals. Tachyphylaxis developed for the first 15 to 25 minutes, but thereafter constant submaximal responses to fixed weights of 5-HT were demonstrated, and the preparations proved satisfactory for assay purposes.

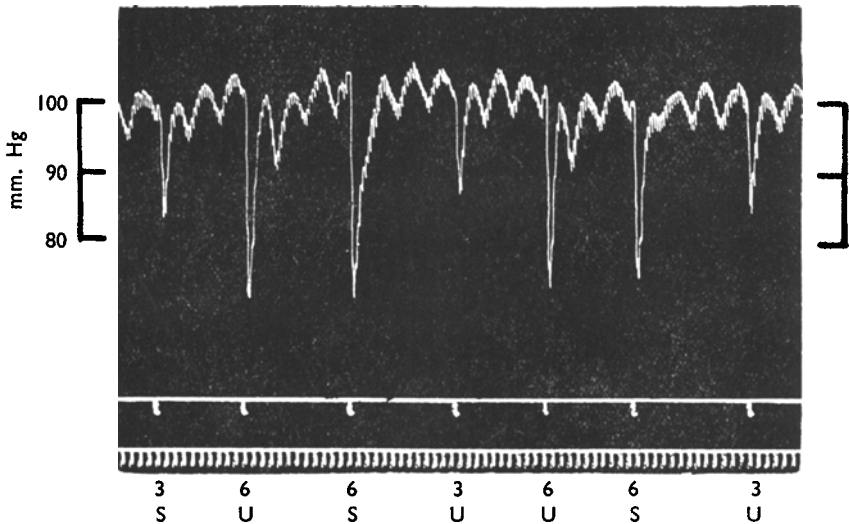


FIG. 4. Part of a record of a 2 + 2 assay of 5-hydroxytryptamine on the blood pressure of a fowl, wt. 1.3 kg. Injections of either standard, S, or test, U, solutions were made i.v. at 3 minute intervals. Doses in ml. $\times 10$.

Figure 4 shows part of a typical tracing obtained during one of these 2 + 2 assays of 5-HT. In this assay both standard and test solutions contained $10 \mu\text{g}$. 5-HT/ml. Twelve groups, each of four randomised doses, were employed. The concentration of 5-HT found in the test solution by experiment was $10.2 \mu\text{g}/\text{ml}$. The Fiducial limits of this mean ($P = 0.95$) were 9.75 to $10.8 \mu\text{g}/\text{ml}$. The value obtained for b was 45.8, and for s/b , 0.02. Evidently, on the blood pressure of the fowl, assays of 5-HT are more precise than assays of histamine.

Adrenaline and noradrenaline. The pressor actions of adrenaline and noradrenaline on the fowl's blood pressure were compared in three assays. In these both the concentrations of adrenaline in the test solutions and of noradrenaline in the standard solutions were known. The dose effect curves for the two drugs did not differ significantly from parallel. $1 \mu\text{g}$. (\pm)-noradrenaline was found equally effective with 1.17 ± 0.08 (3) μg . ($-$)-adrenaline. The precision of these assays were greater than those of histamine, and less than those of 5-HT on the fowl's blood pressure.

REFERENCES

1. Condon, *Bull. Science Techn. Assn.*, 1952, Nov.
2. Schild, *J. Physiol.*, 1942, **101**, 115.