J. Pharm. Pharmac., 1973, **25**, 809–814 *Received May* 29, 1973

The 5-hydroxytryptamine content of rabbit hearts and its release during *in vitro* perfusion

J. R. FOZARD AND G. M. P. MWALUKO

Department of Pharmacology, Materia Medica and Therapeutics, University of Manchester, M13 9PT, U.K.

Rabbit hearts were perfused *in vitro* at constant pressure by the Langendorff technique. After 30 min perfusion, hearts from untreated animals contained significantly more 5-HT than those from animals pretreated with heparin. The cardiac 5-HT content of the non-heparinized group decreased during a further 55 min perfusion and the amount lost from the hearts was equal to that recovered from the perfusion fluid. In contrast, the 5-HT content of hearts from heparinized animals did not change significantly during this period, and no 5-HT could be detected in the perfusates. Investigation of the distribution of 5-HT within the hearts from heparinized animals disclosed a significantly greater concentration of 5-HT in the right atrium than in the rest of the heart. It is concluded that the small, perfusion-resistant store of 5-HT within hearts from heparinized rabbits represents the true endogenous 5-HT content in this species, but that much higher values may be obtained unless care is taken to exclude a contribution from blood platelets.

During recent experiments in which the uptake of 5-HT by the rabbit heart was measured *in vitro* (Fozard & Mwaluko, 1973), an unexpected, but consistent observation was the presence of a small amount of 5-hydroxytryptamine (5-HT) in the fluid leaving the venous side of the heart during perfusion with drug-free Tyrode solution. This phenomenon has now been further investigated, and the present report describes data implicating blood platelets as the source of the 5-HT found in the perfusion fluid. In addition, the existence of a small, perfusion-resistant store of 5-HT within rabbit heart tissue is reported, which, it is suggested, represents the true endogenous cardiac 5-HT content in this species.

MATERIALS AND METHODS

Perfusion of the heart. Male rabbits of unselected strain, 1·5 to 2·5 kg, were first weighed and in some cases given 500 units kg^{-1} of heparin into a marginal ear vein. Two to five min later they were killed by a blow to the head and cervical bleeding. Hearts were rapidly removed and perfused according to the Langendorff technique with modified Tyrode solution at 36°. The Tyrode solution (concentrations in g $litre^{-1}$: NaCl 8·0; KCl 0·2; $CaCl_2$ 0·2; $MgCl_2$ 0·1; $NaHCO_3$ 1·0; NaH_2PO_4 0·05; glucose 1·0; ascorbic acid 0·01) was gassed with a mixture of 5% CO_2 in oxygen. Perfusion pressure was maintained at 60 cm water. After a 30 min equilibration period, the fluid leaving the heart was collected in 7 aliquots (0–2, 2–4, 4–6, 6–10, 10–25, 25–40, 40–55 min). A 12 ml sample from each aliquot was removed for assay of 5-HT. Two additional samples of the perfusion solution were taken from the inflow cannula at the beginning and at the end of the perfusion period and assayed for 5-HT. These served as the perfusate blanks. At the end of the collection period,

hearts were removed from the cannula, trimmed of elastic tissue and fat, cut into small pieces, firmly blotted and weighed.

Extraction and assay of 5-HT. Tyrode samples were immediately added to 0·066 ml ethylenediaminetetra-acetic acid disodium salt (100 mg ml^{-1}) and 0·066 ml freshly-prepared ascorbic acid solution (10 mg ml^{-1}) in a pre-cooled glass drug bottle. The contents were mixed by inversion and immediately cooled in melting ice. Weighed heart tissue was transferred to a pre-cooled, 100 ml centrifuge tube containing 8 ml 0·4 N perchloric acid, 0·2 ml ethylenediaminetetra-acetic acid disodium salt (100 mg ml^{-1}) and 0·2 ml ascorbic acid (10 mg ml^{-1}), and homogenized for 1 min with an Ultra Turrax homogenizer. After centrifugation at 900 *g* for 10 min, the supernatant was decanted and two 2 ml aliquots were removed for assay.

The 5-HT content of perfusates and heart extracts was assayed fluorimetrically by the ninhydrin condensation method of Snyder, Axelrod & Zweig (1965). With this method, about 5 ng of 5-HT could easily be detected in a sample, and amounts greater than 8 ng could be accurately assayed (Mwaluko, 1973). Appropriate standards were made up in Tyrode solution and carried through the entire extraction and assay procedure. The activation and emission fluorescence spectra of the material extracted from rabbit hearts were identical to those obtained with these authentic 5-HT standards. Heart tissue blanks were prepared by the procedure of Contractor (1964). Using this method, duplicate samples of the heart extract were assayed, but with 1 drop of hydrogen peroxide (10 vol) being added to one of the samples just before heating with ninhydrin.

Statistical Analysis

Students *t*-test was used to investigate the significance of a difference between mean values. *P* values less than 0·05 were considered significant. n is the number of individual values making up the mean.

Drugs and Chemicals

Ascorbic acid (British Drug Houses); ethylenediaminetetra-acetic acid disodium salt (British Drug Houses); heparin (Evans Medical); hydrogen peroxide (British Drug Houses); ninhydrin (British Drug Houses); perchloric acid (British Drug Houses); 5-hydroxytryptamine creatinine sulphate (Koch-Light).

RESULTS

The endogenous 5-HT content of rabbit hearts after the 30 min equilibration period: effects of pretreatment with heparin (500 units kg^{-1} intravenously, 2 to 5 min before killing). 1 to 2 min after commencing perfusion, the fluid leaving hearts taken from both non-heparinized and heparinized animals ran visibly free of blood and remained clear throughout the perfusion period. After the 30 min equilibration period, the 5-HT content of hearts taken from non-pretreated animals was $319{\cdot}6 \pm 109{\cdot}4$ ng g^{-1} heart wet weight ($n = 5$). In hearts taken from animals pretreated with heparin it was $70{\cdot}8 \pm 15{\cdot}7$ ng g^{-1} ($n = 5$). The difference is significant ($P < 0{\cdot}05$). Small thrombi could often be seen during preparation of the hearts from non-heparinized animals for assay. Such thrombi were rarely seen in hearts from pretreated animals.

The time-dependent release of 5-HT from rabbit hearts into the perfusion fluid: effects of pretreatment with heparin. The results are presented in Fig. 1. Hearts taken from non-heparinized animals released 5-HT into the perfusion fluid. When expressed

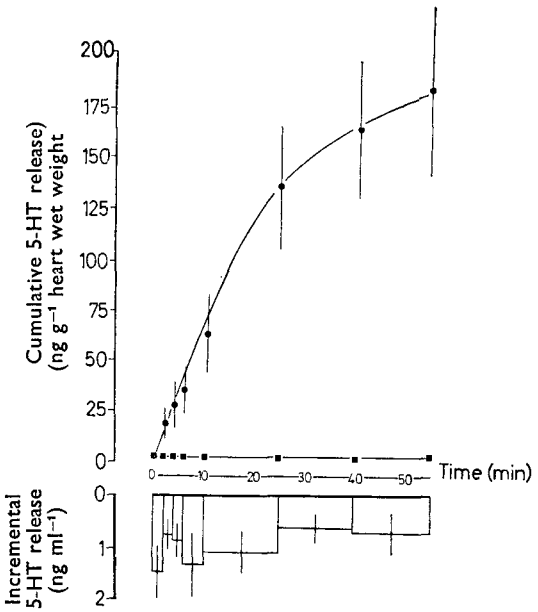

FIG. 1. The time-dependent release of 5-HT into the fluid perfusing rabbit hearts. Upper part, cumulative release in ng g^{-1} heart wet weight; lower part, incremental release in ng ml^{-1}. ●—● hearts from non-heparinized animals (n = 6). ■—■ hearts from animals given heparin 500 units kg^{-1} just before killing (n = 4). Vertical lines indicate the standard errors about the mean values. Note that the incremental release histograms pertain only to the non-heparinized group, since no release occurred in hearts from heparinized animals. Zero time refers to the end of the 30 min equilibration period.

cumulatively (as ng g^{-1}, heart wet weight) and plotted against time, the rate of release declined slowly during the course of the perfusion. The incremental release was highly variable (indicated by the large standard errors about the mean values in Fig. 1), not only for a particular heart, but also between hearts. The total quantity of 5-HT released into the perfusion fluid during the perfusion period was 184·2 ± 41·9 ng g^{-1} (n = 6). When the heart 5-HT content was assayed at the end of the perfusion period it was found to be 139·2 ± 39·2 ng g^{-1} (n = 5), which is less than half the value obtained immediately after the 30 min equilibration period which was 319·6 ± 109·4 ng g^{-1} (n = 5). The loss of 5-HT from these hearts, obtained by subtracting these values, was 180·4 ng g^{-1}, which compares closely with the 184·2 ± 41·9 ng g^{-1} recovered from the perfusion fluid during this time period.

In contrast, in four experiments, no resting release of 5-HT could be detected from hearts taken from animals pretreated with heparin (Fig. 1). Furthermore, the heart 5-HT content after the perfusion period (67·8 ± 1·9 ng g^{-1}, n = 7) was similar to and not significantly different from the value obtained after the 30 min equilibration period (70·8 ± 15·7 ng g^{-1}, n = 5).

The regional distribution of 5-HT in hearts from heparinized animals perfused for the 30 min equilibration period. In these experiments, pooled tissues from two hearts were used for each experimental determination. The hearts were perfused for the 30 min equilibration period, and then carefully dissected into the four chambers which were subsequently assayed for their 5-HT contents. The results are shown in Fig. 2. The 5-HT content of the right atrium was significantly greater ($P < 0·05$) than that of the

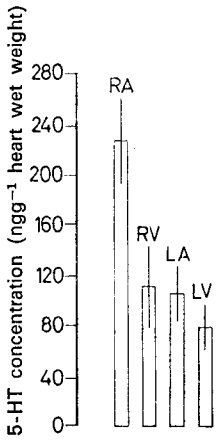

FIG. 2. The regional distribution of 5-HT in hearts from heparinized animals perfused for the 30 min equilibration period. RA = right atrium, RV = right ventricle, LA = left atrium, LV = left ventricle. The right atrial 5-HT content was significantly different ($P < 0{\cdot}05$) from the contents of the other chambers. Other details as Fig. 1.

left atrium and the right and left ventricles. The mean overall 5-HT content of these hearts was $89{\cdot}2 \pm 19{\cdot}9$ ng g^{-1} ($n = 3$).

DISCUSSION

Hearts taken from non-heparinized animals contained significantly more 5-HT after 30 and 85 min perfusion with Tyrode solution than did those from heparinized animals. Since the major property of heparin under the conditions of these experiments is to prevent blood clotting (Goodman & Gilman, 1970), it seems likely that this difference reflects the presence or absence of thrombi in the cardiac tissue. Such thrombi would presumably entrap platelets, and as rabbit platelets are particularly rich in 5-HT (Humphrey & Jaques, 1954; Woolley & Edelman, 1958), this could make a significant contribution to the measured 5-HT content. Certain of the other observations would agree with this suggestion.

Thus, the endogenous 5-HT contents of hearts from non-heparinized animals were markedly variable as can be inferred from the large standard errors about the mean values. This could arise from a random occurrence of thrombi which would be expected from differences in the speed and efficiency with which the heart was removed from the animal and perfusion commenced. In contrast, the individual endogenous 5-HT contents of hearts from heparinized animals were proportionately much less variable, although the setting-up procedure was identical.

Then, the hearts from non-heparinized animals were found to release detectable amounts of 5-HT into the perfusates (Fig. 1) and at the same time their endogenous 5-HT contents decreased. This indicates that the hearts are unable to replace the 5-HT which is lost and accords well with trapped platelets being the source of 5-HT, since platelets cannot themselves synthesize 5-HT, (Gaddum & Giarman, 1956), but rely on active accumulation from the surrounding fluid to maintain their levels (Hughes & Brodie, 1959; Stacey, 1961).

It can also be inferred that the 5-HT appearing in the perfusates derives from intact platelets being washed from the heart tissue rather than a steady leakage of 5-HT from tissue-fast platelets with subsequent transport in the perfusion fluid to the venous

side of the heart. Thus the very variable incremental release from individual hearts is more complementary to the former view than to the latter. Furthermore, determination of the total amount of 5-HT appearing in the perfusion fluid and that disappearing from the heart over the same time period indicated that almost all the 5-HT lost from the heart was recovered intact. This situation could only obtain if the 5-HT was released from the heart in a form protected from tissue uptake and metabolic breakdown processes (Airaksinen, 1963; Fozard, 1969a; Fozard & Mwaluko, 1973). That form could be the intact platelet.

In contrast, the much smaller, endogenous, 5-HT levels of hearts from heparinized animals was not significantly altered by prolonged perfusion with Tyrode solution, and there was no detectable release of 5-HT from these hearts into the perfusates (Fig. 1). The few reports in the literature of the endogenous 5-HT concentrations in rabbit hearts are in accord with the present work. Thus, Waalkes & Coburn (1959), who made no attempt to exclude a contribution from blood platelets, found 400 ng g^{-1}, heart wet weight. Garven (1956) on the other hand quotes a figure of 23 ng g^{-1}. Although Garven did not pretreat her animals with heparin, the hearts were perfused with saline *in situ*, during anaesthesia with ether, to ensure the optimal removal of their blood content before assay. It therefore seems likely that a figure of 69·1 $\pm$ 6·2 ng g^{-1} heart wet weight, n = 12 (obtained by combining the data obtained after 30 and 85 min perfusion of hearts from heparinized animals) reflects the true endogenous cardiac 5-HT content for the rabbit. The results also suggest that in this species, much higher values than this may easily be obtained unless care is taken to exclude a contribution from blood platelets.

The question arises as to where in the heart the small perfusion-resistant store of 5-HT is located. Any potential storage site would have to be compatible with the observations presented in Fig. 2, disclosing a significantly greater 5-HT content in the right atrium than in the other heart chambers. Two obvious sites which should be considered are mast cells and chromaffin cells.

Although many investigators have demonstrated important amounts of 5-HT in mast cells from rats and mice (Garattini & Valzelli, 1965; Erspamer, 1966), none could be detected in rabbit mast cells (Parratt & West, 1957). In any case there seems to be no logical reason for a greater mast cell density in the right atrium than in the rest of the heart, which would have to be the case if the present results were to be supported. Chromaffin cells have been demonstrated in the mammalian heart (Truex, 1950; Jacobowitz, 1967), although no evidence seems to be available for the rabbit, and in no case has it been demonstrated that cardiac chromaffin cells contain 5-HT. However, in some species, chromaffin cells are preferentially located around atrial parasympathetic ganglia (Jacobowitz, 1967), a situation which would, if repeated in the rabbit, give an uneven distribution similar to that shown in Fig. 2.

The distribution of 5-HT in hearts from heparinized animals strongly resembles the distribution of noradrenaline in rabbit hearts (Muscholl, 1959; Angelakos, Fuxe & Torchiana, 1963), and a good correlation exists between the noradrenaline content of a particular region and the density of its noradrenergic innervation as disclosed by histochemical fluorescence microscopy (Angelakos & others, 1963). On this basis, it is tempting to speculate that 5-HT too might be stored in association with sympathetic noradrenergic neurons. Such a suggestion would accord with the results from a number of investigators who have demonstrated an affinity of 5-HT for the binding sites on the noradrenaline storage granules of cardiac sympathetic nerves (Andén, 1964;

Gillis, 1964; Snyder, Wurtman, & others, 1964; Jester & Horst, 1972). It would also have a precedent in that 5-HT has been demonstrated to occur naturally within the sympathetic nerves of the pineal gland of certain species (Owman, 1964).

Acknowledgement

This work was supported in part by the Migraine Trust.

REFERENCES

AIRAKSINEN, M. M. (1963). *Ann. Med. exp. Fenn.*, **41**, Suppl. 4.

ANDÉN, N. E. (1964). *Acta. pharmac. tox.*, **21**, 59–75.

ANGELAKOS, E. J., FUXE, K. & TORCHIANA, M. L. (1963). *Acta physiol. scand.*, **59**, 184–192.

CONTRACTOR, S. F. (1964). *Biochem. Pharmac.*, **13**, 1351–1357.

ERSPAMER, V. (1966). *5-Hydroxytryptamine and Related Indolealkylamines*, p. 141. Berlin: Springer.

FOZARD, J. R. (1969a). *Eur. J. Pharmac.*, **7**, 248–257.

FOZARD, J. R. (1969b). *J. Pharm. Pharmac.*, **21**, 550–551.

FOZARD, J. R. & MWALUKO, G. M. P. (1973). *Br. J. Pharmac.*, **47**, 661P–662P.

GADDUM, J. H. & GIARMAN, N. (1956). *Br. J. Pharmac. Chemother.*, **11**, 88–92.

GARATTINI, S. & VALZELLI, L. (1965). *Serotonin, p.* **56**, London: Elsevier.

GARVEN, J. D. (1956). *Br. J. Pharmac. Chemother.*, **11**, 66–70.

GILLIS, C. N. (1964). *J. Pharmac. exp. Ther.*, **146**, 54–62.

GOODMAN, L. S. & GILMAN, A. (1970). *The Pharmacological Basis of Therapeutics*, 4th edn., p. 1447. London: Collier-Macmillan.

HUGHES, F. B. & BRODIE, B. B. (1959). *J. Pharmac. exp. Ther.*, **127**, 96–102.

HUMPHREY, J. H. & JAQUES, R. (1954). *J. Physiol.*, Lond., **124**, 305–310.

JACOBOWITZ, D. (1967). *J. Pharmac. exp. Ther.*, **158**, 227–240.

JESTER, J. & HORST, W. D. (1972). *Biochem. Pharmac.*, **21**, 333–338.

MUSCHOLL, E. (1959). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **237**, 350–364.

MWALUKO, G. M. P. (1973). M.Sc. Thesis, University of Manchester.

OWMAN, Ch. (1964). *Acta physiol. scand.*, **63**, Suppl 240.

PARRATT, J. R. & WEST, G. B. (1957). *J. Physiol.*, Lond., **137**, 169–178.

SNYDER, S. H., AXELROD, J. & ZWEIG, M. (1965). *Biochem. Pharmac.*, **14**, 831–835.

SNYDER, S. H., WURTMAN, R. J., AXELROD, J. & CHU, E. W. (1964). *J. Pharmac. exp. Ther.*, **146**, 276–279.

STACEY, R. S. (1961). *Br. J. Pharmac. Chemother.*, **16**, 284–295.

TRUEX, R. C. (1950). *Anat. Rec.*, **108**, 687–697.

WAALKES, T. Ph. & COBURN, H. (1959). *J. Allergy*, **30**, 394–407.

WOOLLEY, D. W. & EDELMAN, P. M. (1958). *Science*, **127**, 281–282.