THE RESPONSE OF ISOLATED FROG HEARTS TO MINIMUM EFFECTIVE CONCENTRATIONS OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE

C. L. PATHAK

Department of Physiology, Medical College, Jodhpur

Summary : The action of increasing concentration of adrenaline, noradrenaline and 5-hydroxytryptamine on cardiac output, arterial pressure and rate of isolated perfused frog hearts working against a fixed resistance was investigated. The minimum effective concentration varied from heart to heart and ranged from 10^{-9} to 10^{-5} for catecholamines and from 10^{-9} to 10^{-3} g/ml for 5-hydroxytryptamine. The least effective concentration of 5-hydroxytryptamine (10^{-9}) was 100 times more than the least effective concentration of catecholamines (10^{-11}). Both inotropic and chronotropic responses were simultaneously affected at the least effective concentration and at greater concentrations. However the first effect on inotropic response was more common than on chronotropic response.

Noradrenaline produced stimulation in 100% cases while adrenaline and 5-hydroxytryptamine produced excitatory as well as inhibitory effects. Biphasic effects were observed in the forms of stimulation and inhibition occurring separately at different concentrations in the same heart or simultaneously at some concentration. Direct biphasic effect of 5-HT may explain complex changes in blood pressure observed in intact animals by previous workers. Inhibitory and biphasic effects of 5-HT are reported for the first time.

Key Words : 5-hydroxytryptamine, catecholamines, biphasic cardiac actions, isolated frog heart, cardioinhibition by 5-HT

A high degree of sensitivity of isolated frog hearts to acetylcholine has already been reported, the minimum effective concentration in some hearts being as small as 10^{-19} *g/ml.* (4,5,30). The detection of very small changes in several parameters *i.e.* cardiac output, arterial pressure, heart rate and venous pressure was made possible with the help of a specially designed experimental set up, a four channel recorder and other electronic assemblies. It was considered desirable to investigate the sensitivity of the frog hearts to other neurohumoral transmitters. This paper reports the response of isolated frog hearts to widely separated but serially increasing concentrations of adrenaline, noradrenaline and 5-hydroxytryptamine.

MATERIALS AND METHODS

Full details of experimental arrangement have been published elsewhere (4, 5). The heart of pithed frog was perfused through the posterior vena cava with Ringer's solution (Na Cl, 108.7 mM; KCl, 3.8 mM; Ca Cl_2, 1.6 mM; Na H Co_3, 4.3 mM; $NaHPO_4$, 0.22 mM; Glucose, 5.0 mM) of pH 7.8 to 8.0. The outflow from the two aortae was lead through a common artificial resistance of a desired value to give arterial pressures roughly equivalent to

those in intact animals *i.e.* 20 to 40 mm Hg. The cardiac output (outflow from resistance during steady state), arterial pressures, heart rate and perfusion pressure (venous pressure) were measured electronically and displayed continuously on a 4 channel direct ink writing recorder with servo-operated pens. Vigorous precautions were taken to eliminate or control all sources of contamination including toxicity arising from the use of connecting tubing (6).

Adrenaline was supplied by B.D.H. in sealed ampoules of 1 ml of 1 in 1000 concentration. Noradrenaline bitartrate was obtained from Bayer Products Ltd. in sealed ampoules of 2 ml of 1 in 1000 concentration. The supply of 5-hydroxytryptamine was obtained from Roche Products Ltd. in a powder form as serotonin creatinine sulphate. Serial dilutions of these substances in Ringer's solutions were prepared in 100 ml flask by a standard dilution procedure in steps of 1 in 100, starting from 10^{-3} *g/ml* and working upto 10^{-11} *g/ml* or still lower concentration when considered necessary. The test solutions were administered by perfusion, starting with ineffective concentration, each for a period of 2 minutes and the test perfusions were bracketed between control perfusions from the same vessel. Perfusion pressure was also monitered on a sensitive meter and with special motor carriage system it could be kept at a constant value within 0.1 mm of water so as to avoid cardiodynamic changes due to change in perfusion pressure (26, 27, 28). The temperature of the laboratory was controlled thermostatically.

RESULTS

Chronotropic responses were estimated from the record of heart rate and inotropic responses, from the amplitude of pulse pressure and cardiac output. If the response of a substance was inhibitory at one concenration and excitatory at another, or if complex changes occurred at any concentration with evidence of both inhibitory and excitatory components, the action was termed 'biphasic'. The term threshold sensitivity means responsiveness to the minimum effective (threshold) concentration.

The observations of experiments on 26 hearts are summarised in Table I. Because of involvement of different recorded parameters in positive and/or negative direction quantitation of net effect was difficult. In Table I each + or − sign represents a 10% change above control values. The percentages of hearts affected at various concentrations of adrenaline (ADR), noradrenaline (NOR), and 5-hydroxytryptamine (5-HT) are shown in Fig. 1. Table II gives the number of tests in which the inotropic or chronotropic response was first affected irrespective of concentration and the nature of change in the first affected parameter. Fig. 2 gives the percentage of hearts which showed frank stimulation or inhibition or clear biphasic effect at different concentration of the three substances. The observations on the effect of each substance are considered individually.

ADRENALINE

Threshold concentrations : The minimum effective concentration of adrenaline varied

from heart to heart and ranged from 10^{-11} to 10^{-5} *g/ml*. The threshold concentration was 10^{-11} *g/ml* in 1 heart, 10^{-9} *g/ml* in 5 hearts, 10^{-7} *g/ml* in 13 hearts and 10^{-5} *g/ml* in 3 hearts. Thus 19 out of 22 (about 86%) hearts responded to concentrations ranging from 10^{-9} to 10^{-7} *g/ml* (Table 1 and Fig. 1).

TABLE I : Actions at various concentrations in g/ml

Heart No.	*Adrenaline*				*Noradrenaline*				*5-HT*		
	10^{-11}	10^{-5}	10^{-9}	10^{-9}	10^{-11}	10^{-9}	10^{-7}	10^{-5}	10^{-9}	10^{-7}	10^{-5}
1									0	0	+
2									0	+R+	++R+++
3	0	0	+								
4	0	0	+	+	0	0	+	+ +			
5					0	0	0	++ ++	0	0	0
6									0	0	+
7	0	—	+++		0	0	++		0	—R—	— —R+—
8	0	+R+			0	+R+			0	—R++	—R+++
9	+	+	+++		+	+	+ +	+++			
10	0	0	++ ++		0	0	+++	++++			
11	0	0	++ ++		0	0	++ ++				
12	0	0	0		0	0	++ ++				
13	0	0	—R—	+++	0	0	0	+++++	0	0	—+R+++
14	0	0	+ +		0	0	0		0	R+	+—+R+++
15	0	0	0	+++++							
16	0	—R—	— —	++++	0	0	+ +	+++	0	—R+	+—+R++
17	0	0	+++	+++	0	0	0	+++			
18	0	— —	+ +	++	0	0	+R+	++	0	—R+	+
19	0	0	++ +		0	0	+ +		—	— —	— — —
20	0	0	+ +		0	0	+		0	0	+
21	0	0	+++		0	0	+ +		0	0	+
22	0	0	+ +		0	0	+		0	0	+
23	0	0	°°R+	++	0	0	+	+	0	0	0
24	0	0	0	+++	0	0	+	+++++	0	— —	— — —
25	0	0	0	+++	0	0	+	+++	0	0	—R+
26	0	0	++++S		0	0	+++		0	—R+	

For all three variable parameters:—O, no effect, +, increase and —, decrease. S, transient stoppage; R, Heart Rate. Signs (+ or —) prefixed to R indicate change in blood pressure and output associated with change in R as shown by suffixed signs.

Note:— The table does not include actions of 5-hydroxytryptamine (5-HT) 10^{-3} g/ml.

The least effective concentration of adrenaline under present experimental conditions was 10^{-11} *g/ml* equivalent to 4.5×10^{-11} M. At this concentration the heart showed frank excitatory

effect involving both the inotropic and chronotropic responses (Table I, heart 9 & Fig. 1). At higher concentrations greater number of hearts were affected (Fig. 1) till at 10^{-5} g/m hearts, in which this concentration was tested, were affected following a sigmoid relation between involvement-incidence and concentration.

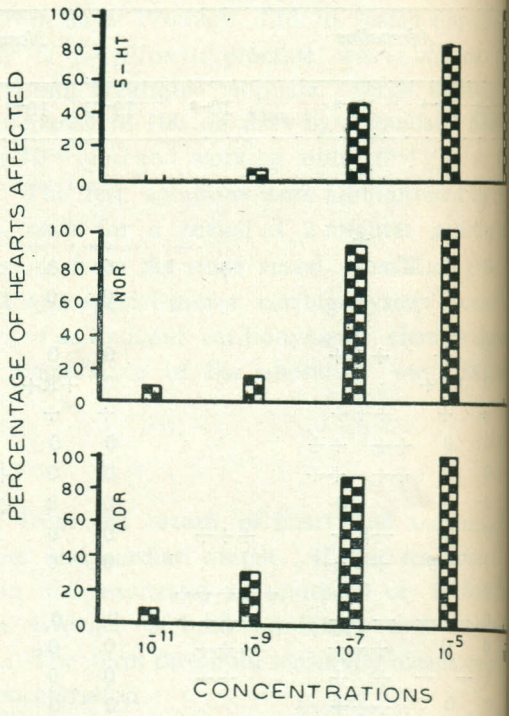

*Fig.*1 : Percentage of hearts affected at various concentrations in g/ml of adrenaline (ADR), noradrenaline (NOR) and 5-hydroxytryptamine (5-HT).

Nature of action: Fig. 2A shows the incidence of stimulatory, inhibitory or biphasic respo ses to increasing concentration of adrenaline and confirms the sigmoid type of relationship in of frank excitatory effect. The curve for the incidence of inhibitory effect was linear decli progressively as the concentration was raised till at 10^{-5} *g/ml* the inhibitory effect was repla by stimulation in all hearts. Significant inhibitory effects occurred in 4 out of 22 (18%) he at low concentration of adrenaline out of these 4 hearts 3 showed the inhibitory actio threshold concentration of 10^{-9} *g/ml* while 1 heart showed inhibitory effects at 10^{-7} g Clear biphasic effect was seen only in heart 26 in which adrenaline at 10^{-7} *g/ml* initially p duced profound stimulation followed by repeated periods of asystole at intervals. I 4 hearts which showed inhibition at low concentrations and stimulation at high concentrati are included in the category of heart showing biphasic effects the over all incidence of biph effect would be 22% (5 out of 22).

TABLE II : Involvement of inotropic and chronotropic responses.

First affected response	*Noradrenaline Total trials=30*			*Adrenaline Total trials=32*			*5-Hydroxytryptmine Total trials=25*		
	Total	+	—	*Total*	+	—	*Total*	+	—
Inotropic	2	2	0	3	1	2	13	4	9
Chronotropic	0	0	0	1	1	0	1	1	0
Both together	28	28	0	28	25	3	11	6	5
TOTAL	30	30	0	32	27	5	25	11	14

Stimulation+, Inhibition—.

Table II shows that the action on inotropic and chronotropic responses was dissociable in some trials and in case of adrenaline the inotropic response was more frequently affected first than the chronotropic response. When affected first, the inotropic response changed positively or negatively. The negative inotropic effect was predominently responsible for the incidence of inhibitory effect. Perhaps the diffusion of adrenaline to pacemaker site is restricted in comparison to the contractile myocardium so that threshold concentration is more likely to involve the contractile musculature. The chronotropic response was positive when it was first affected at the threshold concentration otherwise it was effected in the same way as the inotropic response.

NORADRENALINE

Threshold concentration: The threshold sensitivity to noradrenaline varied from 10^{-11} to 10^{-5} *g/ml*. Only 1 out of 21 hearts responded to 10^{-11} *g/ml* and 2 hearts responded to 10^{-9} *g/ml*. In 15 hearts the minimum effective concentration was 10^{-7} *g/ml* while the first effective concentration in the remaining 3 hearts was 10^{-5} *g/ml*. Thus 18 out of 21 (about 86%) hearts responded to concentration ranging from 10^{-9} to 10^{-7} *g/ml*. The least effective concentration 10^{-11} *g/ml* equivalent to 2.7×10^{-11} M producing frank stimulation of both inotropic and chronotropic responses.

Nature of action: Tables I & II and Figs. 1 and 2 show that the effect of increasing concentrations of noradrenaline was similar to that of adrenaline with the following exceptions:—

(a) The chronotropic response was not involved alone nor it was affected first in any trial.

(b) Only stimulation of both chronotropic and inotropic responses was observed. Unlike adrenaline no inhibitory or biphasic effects were seen.

5—HYDROXYTRYPTAMINE

Threshold concentration: From Table I and Fig. 1 it is at once clear that the minim effective concentration of 5-HT was 100 times greater than that of catecholamines. Thus frog hearts were relatively less sensitive to 5-HT than to catecholamines. The thres

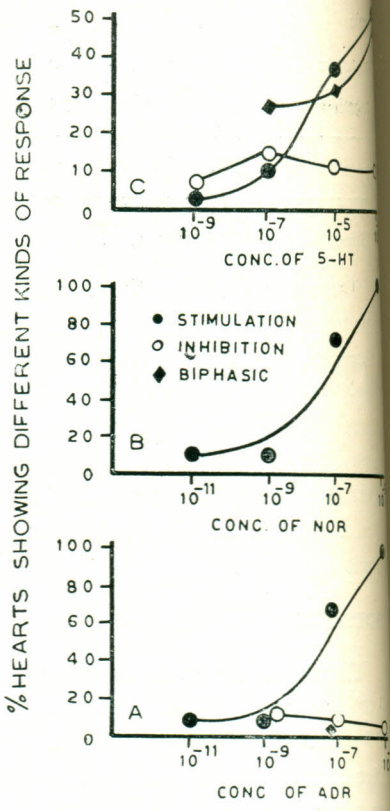

*Fig.*2.: Precentage of hearts showing stimulation, inhibition or biphasic effects at different concentrations in g/ml of A. adrenaline (ADR), B. noradrenaline (NOR) and C. 5-hydroxytryptamine (5-HT).

sensitivity to 5-HT ranged from 10^{-9} *g/ml* to 10^{-3} *g/ml*. Only one out of 18 hearts show threshold sensitivity at 10^{-9} *g/ml*. The minimum effective concentration in 8 hearts w 10^{-7} *g/ml* while 7 heatrs responded to a concentration as high as 10^{-5} *g/ml*. The remaini 2 hearts responded to 10^{-3} *g/ml*. Thus 16 out of 18 hearts (about 88%) responded to conce trations ranging from 10^{-7} to 10^{-5} *g/ml*.

The least effective concentration was 10^{-9} *g/ml* equivalent to 4.2×10^{-10} M whi produced frank inhibitory effect (Table I, heart 19). The incidence of involvement of hearts increasing concentrations increased in a linear fashion unlike catecholamines which show sigmoid pattern (Fig. 1).

Nature of action: Fig. 2 shows that the stimulatory action of 5-HT followed a sigmoid curve as in case of catecholamines. The incidence of inhibitory effects was maximum at 10^{-7} *g/ml* but occurred at all concentrations. Biphasic effects were common with 5-HT at concentrations above 10^{-7} *g/ml* and the incidence of biphasic effects was almost equal to the incidence of excitatory effects at 10^{-3} *g/ml*. Complex changes in blood pressure and output records were seen due to biphasic effects. Graded inhibitory effects at all concentration were recorded in 10% hearts. Table II shows that in almost half the test with 5-HT the inotropic response was first affected. The incidence of negative inotropic effects was twice more common than the incidence of positive inotropic effect when the inotropic was first involved. The incidence of involvement of inotropic and chronotropic response individually paralled that observed in case of adrenaline. However when both inotropic and chronotropic responses were simultaneously affected, half the trials showed excitation and the remaining half were characterized by inhibitory effects unlike adrenaline which produced stimulation in 25 out of 28 hearts. The chronotropic response was affected first only in heart 14 (Table I) in which case acceleration occurred. The positive chronotropic action of 5-HT was quite prominent in many hearts (Table I).

From the above results it is clear that:—

(1) The least effective concentration of catecholamines was 10^{-11} *g/ml* but the threshold concentration varied from heart to heart. The least effective concentration of 5-HT was 100 times more i.e. 10^{-9} *g/ml*.

(2) Involvement of chronotropic response alone or first involvement of chronotropic response was not common and wherever the chronotropic response was so involved, acceleration was observed.

(3) Noradrenaline produced only excitatory effect at all concentrations. Adrenaline and 5-HT produced either excitatory or inhibitory or biphasic effects. The incidence of biphasic effects with adrenaline at the same concentration was rare but about 10 to 15% hearts showed inhibition at low concentrations and excitation at high concentrations. In case of 5-HT, the incidence of biphasic effect increased with concentration.

(4) The incidence of inhibitoty effects with adrenaline and 5-HT was less at high concentration.

(5) The commonest effect with all three substances was involvement of inotropic and chronotropic responses together. Negative inotropic effects largely contributed to the inhibitory component of biphasic effects.

(6) The incidence of stimulation was 100% in case of noradrenaline, very high in case of adrenaline while with 5-HT stimulation or inhibition plus biphasic action occurred with almost equal frequency.

DISCUSSION

The solutions of progressively increasing concentration were tested in steps of dilution of 1 in 100. This procedure permitted the testing of a wide range of concentrations without subjecting the heart to too many tests. The tests solutions were administered by perfusion for 2 minutes. The cardiac output was about 2 *ml* per min. Hence about 4 *ml* of test solution reached the heart under normal conditions. The total dose can be calculated fairly accurately by multiplying: Cardiac output, testing time and concentration. In usual pharmacological work the doses are administered by injecting test solutions in the perfusion system and it is very difficult to know the actual acting concentration.

Since solutions were tested in steps of 1 in 100 dilution, the minimum effective concentration observed was not the real threshold concentration, the value of which obviously lies between the ineffective and the first effective concentration. Repetitions of tests with smaller steps of dilution in order to define the real minimum effective concentration was not considered desirable, at this stage, in the middle of the experiment. However, the observed values appear to be very close to the real threshold concentrations because the observed minimum effective concentration was associated with quite small effect.

Threshold and least effective concentrations: The sensitivity of frog heart to adrenaline and noradrenaline was almost equal, while the frog heart was 100 times less sensitive to 5-HT. The least effective concentration of adrenaline and noradrenaline was 10^{-11} *g/ml* while that of 5-HT was 10^{-9} *g/ml*. The variation in sensitivity from heart to heart may be due to diffusion parameters, inactivating mechanisms, distribution of receptors or receptor characteristics like storage.

West (38,39) used frog hearts for assay of catecholamines only upto 10^{-8} *g/ml* dilution. Greater dilutions have been found effective on pressor response in rats (9), rat uterus and rat colon (15, 16). The sinsitivity to 5-HT has been tested in a number of preparations. The isolated colon of rat (10,14), isolated rat uterus (3) and fundal strips of rat stomach (36) have been reported to be sensitive to 5-HT upto 10^{-9} *g/ml*. Hearts of marine invertebrates are more sensitive to 5-HT which stimulates them (12, 17, 35, 37) upto a dilution of 10^{-10} *g/ml*.

Nature of action: The excitatory actions of catecholamines are brought about presumably through adrenergic beta receptors (1,2) through the formation of cyclic nucleotide which activates phosphorylase to increase glycolysis (34). Adrenaline is the dominent neurohormone in hearts (13).

The inhibitory effects of adrenaline, however, have not received enough attention although many workers have reported inhibitory effects from adrenaline in the isolated heart of frog (18, 19, 33), fish (8) and molluscs (17). The present work further confirms the occurrence of difinite inhibitory effects of adrenaline even at low concentration. These inhibitory effects cannot be attributed to artefacts or decomposition product as the tests were well controlled

and with the present technique of using all fresh clean sterile glassware the strengths of solutions was well preserved. Also there was no correlation between the duration of standing of solutions and the incidence of inhibitory effects. The interpretation of physiological significance of inhibitory effects of adrenaline has been difficult. However the observation during the present study that adrenaline involves mainly the inotropic response during inhibitory effect may facilitate understanding.

The occurrence of inhibitory effect at one concentration and excitatory effect at another concentration in the same heart with the same substance or the simultaneous occurrence of inhibitory and excitatory affect at one concentration could only occur when the acting substance could involve common receptor mechanism both positively and negatively. Such biphasic effects could also occur even if there were separate excitation coupled and inhibition coupled receptors provided both are involved by the acting substance. In any case the substance has to have bidirectional affinity. This is why the apparently two phenomena i.e. occurrence of inhibition and stimulation at different concentration in the same heart or simultaneous occurrence of inhibition and excitation leading to complex change at any concentration, both have been put under the category of biphasic effects.

Excitatory effects of 5-HT involving both inotropic and chronotropic responses in marine invertebrate hearts (12, 15, 35, 37) and several species of mammalian hearts (32) have been reported by previous workers. In the present study the excitatory action of 5-HT was observed to be more prominent on chronotropic response. This is consistent with the finding of other workers on mammalian hearts (23, 24).

Inhibitory or biphasic actions of 5-HT were not observed by previous workers either in vertebrate or invertebrate hearts. The complex changes in blood pressure observed by other workers (22, 23. 24, 31) in intact preparations were attributed to coronary chemoreflex (11, 19) or increased arterial resistance (20) or ganglionic blockade (25). The observation of complex changes in cardiac output and blood pressure in isolated hearts working against a fixed resistance rules out involvement of arterial resistance or ganglionic blockade. Coronary chemoreflex is not operative in frogs. Hence the complex changes in intact mammals observed by previous workers are attributable to direct biphasic action 5-HT on the heart.

REFERENCES

1. Ahlquist, R.P. A study of adrenotropic receptors. *Am. J. Physiol.*, **153** : 586-599, 1948.
2. Ahlquist, R.P. 'Adrenergic drugs'. In 'Pharmacology in Medicine', New York, *Mc Graw-Hill*, Chap. 27, 1958.
3. Amin, A.H., T.B.B. Crawford and J.G.H. Gaddum. The distribution of substance P and 5-hydroxytryptamine in the central nervous system of dog. *J. Physiol.*, **126** : 596-618, 1954.
4. Boyd, I.A. and C.L. Pathak. The response of perfused frog hearts to minute quantities of acetylcholine and the variation in sensitivity with season. *J. Physiol.*, **176** : 191-204, 1965.
5. Boyd, I.A. and C.L. Pathak. The action of anticholinesterases on the response of the pacemaker, the conducting system and the ventricular musculature of perfused frog hearts to acetylcholine. *Quart. J. Exp. Physiol.*, **50** : 330-346, 1965.
6. Boyd, I.A. and C.L. Pathak. The comparative toxicity of silicone rubber and plastic tubing. *Scot. Med. J.*, **4** : 345-351, 1964.
7. Burn, J.H. and D.E. Hutcheon. Action of noradrenaline. *Brit. J. Pharmacol.*, **4** : 373-380, 1949.

8. Claes, E. Action de l' extrait surrenal et de l' adrenaline sur le cocur isole du lapin. *Arch. int. Physiol.* **22** : 322-343, 1924.

9. Crawford, T.B.B. and A.S. Outschoorn. Quantitative separation of adrenaline and noradrenaline in biological fluids and tissue extracts. *Brit. J. Pharmacol.*, **6** : 8-19, 1951.

10. Dalgleish, C.E., C.C. Toh and T.S. Work. Fractionation of the smooth muscle stimulant present in extracts of gastrointestinal tract; identification of 5-hydroxytryptamine and its distinction from substance. *P.J. Physiol.* **120** : 298-310, 1953.

11. Dawes, G.S. and J.H. Comroe. Chemo-reflexes from the heart and lungs, *Physiol. Rev.*, **34** : 167-201, 1954.

12. Erspamer, V. and G. Boretti. Isolation and characterization by paper chromatography of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of octopoda and in other tissue extracts of vertebrates and invertebrates. *Arch. int. Pharmocodyn.*, **88** : 296-332, 1951.

13. Euler, V.S. Von. "Noradrenaline". Springfield, Illinois, Charles *Thomas Publications*, 1956.

14. Feldberg, W. and C.C. Toh. Distribution of 5-hydroxytrypramine (serotonin, enteramine) in the wall of the digestive tract. *J. Physiol.*, **119** : 352-362, 1953.

15. Gaddum, J.H. Estimation of substance liberated by adrenergic nerves. *Methods Med. Res.*, **3** : 116-313, 1950.

16. Gaddum, J. H. and F. Lembeck. The assay of substances from the adrenal medulla. *Brit. J. Pharmacol.*, **4** : 401-408, 1949.

17. Gaddum, J.H. and N.K. Passonen. The use of some mulluscan heart for the estimation of 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **10** : 478-485, 1955.

18. Kolm., R. and E.P. Pick. Uber de Bedeutung des calciums fur die Erregbarkeitder sympathischen Herznervenen-digungen. Pflugers *Arch. ges. Physiol.*, **189** : 137-143, 1921.

19. Kottegoda, S.R. and J.C. Mott. Cardiovascular and respiratory actions of 5-hydroxytryptamine in the cat. *Brit. J. Pharmacol.*, **10** : 66-72, 1955.

20. Mac Cannon, D.M. and S.M. Horvath. Some effects of serotonin in pentobarbital anaesthetised dog. *Am. J. Physiol.*, **179** : 131-134, 1954.

21. Mac Donald, A.D. Action of adrenaline on perfused fish heart. *Quart. J. exp. Physiol.*, **25** : 60-80, 1925.

22. Outschoorn, A.S. and J. Jacob. A study of antagonists of 5-hydroxytryptamine and catecholamines on the rat's blood pressure. *Brit. J. Pharmacol.*, **15** : 131-139, 1960.

23. Page, I.H. "5-Hydroxytryptamine". London, *Pergamon*, 1957.

24. Page, I.H. Serotonin (5-hydroxytryptamine) : the last 4 years. *Physiol. Rev.*, **38** : 277-335, 1958.

25. Page, I.H. and J.W.Mc Cubbin. Arterial pressure response to infused serotonin in normo-tensive dogs, cats, hypertensive dogs and man. *Am. J. Physiol.*, **184** : 265-270, 1956.

26. Pathak, C.L. The influence of stretch stimuli on the chronotropic response of the frog's isolated whole heart and its individual chambers. *Indian J. Med. Sci.*, **11** : 808-812, 1957.

27. Pathak, C.L. The effect of stretch on formation and conduction of electrical impulses in the sinoaurcular chamber of frog's heart. *Am. J. Physiol.*, **192** : 111-113, 1958.

28. Pathak, C.L. Effect of changes in intraluminal pressure on inotropic and chronotropic responses of isolated mammalian hearts. *Am. J. Physiol.*, **194** : 197-199, 1958.

29. Pathak, C.L. The influence of some autonomic drugs on the activity of the spontaneously beating isolated frog heart and its sinoauricular chamber. *Indian. J. Med. Res.*, **46** : 442-454, 1958.

30. Pathak, C.L. The sensitivity of frog hearts to acetylcholine and other neurohumoral transmitter and allied substances, *Ph.D. Thesis, University of Glasgow*, 1963.

31. Robson, J.M. and R.S. Stacey. "Recent advances in Pharmacology", London, *Churchill*, page 130, 1962.

32. Schneider, J.A. and F.F. Yonkman Species differences in the respiratory and cardiovascular responses to serotonin (5-hydroxtyptamine). *J. Pharmacol.*, **111** : 84-98, 1954.

33. Sollman, T. and O.W. Barrow. The relation of depressant and stimulant action of epinephrine on frog heart. *J. Pharmacol.*, **29** : 233-255.

34. Sutherland, E.W. and T.W. Rall. "Adrenergic Mechanism", *Ciba Found. Symp.* 1960, London, *Churchill*, page 295, 1961.

35. Twarog, B.M. and I.H. Page. Serotonin content of some mammalian tissues and urine and a method for its determination . *Am. J. Physiol.*, **175** : 157-161, 1953.

36. Vane, J.R. A sensitive method for the assay of 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **12** : 344-349, 1957.

37. Welsh, J.H. Excitation of the heart of Venus mercenaria. *Arch. exp. Path. Pharmak.* **219** : 23-29, 1953.

38. West, G.B. A comparative biological assay of activity in simple solutions of adrenaline *J. Physiol.*, **102** : 367-371, 1943.

39. West, G.B. Quantitative studies of adrenaline and noradrenaline. *J. Physiol.*, **106** : 418-425. 1947.