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EVIDENCE FOR NORADRENALINE AND ADRENALINE AS SYMPATHETIC TRANSMITTERS IN THE CHICKEN

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1 The concentrations of noradrenaline and adrenaline in various organs, arterial plasma and venous outflow from isolated hearts of adult chickens have been determined.

2 The relative adrenaline concentrations (percentage of the sum of noradrenaline and adrenaline) in the heart (33%), spleen (16%) and brain (26%) were higher than those found in mammalian organs. Chemical sympathectomy by pretreatment with 6-hydroxydopamine caused a decrease of the noradrenaline and adrenaline concentrations in the heart to 20 and 23% and in the spleen to 16 and 29%, respectively.

3 Stimulation of the right sympathetic nerves, infusion of tyramine or infusion of a modified Tyrode solution containing 108 mM K^+ and 44 mM Na^+ caused an output of both noradrenaline and adrenaline into the perfusate of isolated hearts. The relative adrenaline concentration in the perfusate (20-28%) was not significantly different from the relative adrenaline concentration remaining in these hearts (19-22%). In the individual experiments, the noradrenaline: adrenaline ratios of the stimulation perfusates were positively correlated with the ratios found in the hearts.

4 The effects of noradrenaline and adrenaline on cardiac rate and tension development were studied in spontaneously beating right atria and electrically driven left atria, respectively. In addition, the arterial pressure rise in response to noradrenaline or adrenaline was measured in chickens. It was found that the cardio-vascular potency of noradrenaline, as reflected by the increases in heart rate, cardiac tension development and arterial blood pressure, was not significantly different from that of adrenaline.

5 It is concluded that, in the chicken heart and spleen, both noradrenaline and adrenaline act as sympathetic neurotransmitters.

Introduction

It has been shown that both noradrenaline and adrenaline are present in the brain and peripheral tissues of chicks and chickens (review by Holzbauer & Sharman, 1972). The concentrations of adrenaline found in these tissues are higher than in the corresponding tissues of mammals. Thus the question of whether noradrenaline or adrenaline is the sympathetic transmitter in the chicken has been raised. Callingham & Cass (1966) speculating from indirect evidence, suggested that adrenaline might fulfil this function and that noradrenaline acts only as a precursor. However, Sturkie & Poorvin (1973) obtained direct evidence that noradrenaline is a sympathetic transmitter by showing that sympathetic nerve stimulation caused a release of noradrenaline from the isolated heart of the chicken. As the authors did not detect a release of adrenaline following nerve stimulation, the fate and function of adrenaline present in sympathetically innervated organs remained obscure. Interestingly the same controversy exists amongst histologists using fluorescent histochemical techniques to distinguish the roles of noradrenaline and adrenaline in adrenergic transmission (Bennett & Malmfors, 1970).

Preliminary results of the present work were presented at the meeting of the Deutsche Pharmakologische Gesellschaft (Lindmar &

DeSantis, 1974) and the Second Congress of the Hungarian Pharmacological Society (Löffelholz & Lindmar, 1974).

Methods

Collection of blood and isolation of organs

The chickens used for the experiments were white adult HNL (Heisdorf-Nelson-Lohmann) hens 3-19 months old weighing 1.0-1.8 kg. The hens were anaesthetized with sodium hexobarbitone (40 mg/kg, i.v. into the brachial vein), and the thoracic cage opened so as to reveal the heart. A cannula was inserted into the left ventricle and one blood sample of approximately 30 ml or three samples of 10 ml each were collected in tubes containing heparin (250 i.u. dissolved in 0.2 ml 0.9% w/v NaCl solution (saline)). The exsanguination procedure lasted approximately 1 minute. The heart, spleen, adrenal glands and brain were then removed for estimation of noradrenaline and adrenaline. Cerebellum and cerebral hemispheres were dissected from the brains, leaving the thalamus, hypothalamus, midbrain and medulla behind.

Perfusion of the heart

In the release experiments, the isolated hearts were perfused by the Langendorff technique with Tyrode solution of the following composition (mM): Na^+ 149.3, K^+ 2.7, Ca^{++} 1.8, Mg^{++} 1.05, Cl^- 145.4, HCO_3^- 11.9, $H_2PO_4^-$ 0.4, (+)-glucose 5.6. The solution contained ascorbic acid 10 mg/l and was maintained at 34° C. This solution was gassed with a mixture of 95% O_2 and 5% CO_2. The perfusion apparatus which is described in detail elsewhere (Kuschinsky, Lindmar & Wollert, 1974) allowed a rapid change from one perfusion medium to another.

The hearts were first perfused with a constant pressure (600 mmH_2O) to detect accidental coronary occlusions (e.g. blood clot, gas bubble). The flow rate was 35 ± 2 ml/min (n = 34) at this pressure. Thereafter the hearts were perfused with a constant volume (24-25 ml/min) using a Desaga peristaltic pump. The heart rate was measured by counting the contractions in a 15 s period.

Sympathetic nerve stimulation

In some experiments the right sympathetic nerves were exposed according to Tummons & Sturkie (1969) and isolated up to the sympathetic chain. Then the hearts with the nerves attached were removed and perfused as described above. The nerves were stimulated with platinum ring electrodes connected to a Grass S6 stimulator at 20 Hz (1 ms, 40 V).

Pretreatment with intravenous adrenaline or 6-hydroxydopamine

(−)-Adrenaline (400 μg/kg) was infused by means of a pump over a 40 min period into a brachial vein of chickens anaesthetized with sodium hexobarbitone. During this time anaesthesia was maintained as necessary with ether. The dissection, collection of blood, and removal of organs were performed as already described. Four chickens were pretreated with 6-hydroxydopamine hydrochloride 2 x 20 mg/kg given intravenously 14 days before and 2 x 50 mg/kg 7 days before the experiment; 100 mg was dissolved in 5 ml 0.001 N HCl which was gassed with nitrogen (schedule according to Thoenen & Tranzer, 1968).

Estimation of catecholamine concentrations of organs, plasma and perfusates

Organs were homogenized and extracted in 5% trichloroacetic acid (TCA). Heparinized blood was centrifuged at 0° C for 5 min and the plasma mixed with an equal volume of 10% TCA. After 20 min of extraction the mixture was centrifuged for 5 min and the catecholamines were estimated in the supernatant. The perfusates of the isolated hearts were immediately acidified with 1 N H_2SO_4 to achieve a pH of 3-4.

The catecholamines of tissues, plasma and perfusate were estimated fluorimetrically by the trihydroxindole method after adsorption on, and elution from, aluminium oxide as described previously (Lindmar & Muscholl, 1964). That the amines detected in tissues, plasma and perfusates by this method were, indeed, noradrenaline and adrenaline was established by paper chromatography according to the method described by Muscholl (1959).

Recoveries from a mixture of 200 ng noradrenaline and 50 ng adrenaline were 68 ± 5% for noradrenaline and 67 ± 10% for adrenaline (n = 9) when the mixture was added to the TCA-extract of the chicken heart. Similar recoveries (67 ± 5% for noradrenaline and 62 ± 5% for adrenaline) were obtained when the same mixture (200/50) was added to perfusate samples. It is concluded that under the present conditions any underestimation of the relatively small amounts of adrenaline was not significant. These recovery experiments are particularly important for the evaluation of the present results since it is known that the differential estimation of adrenaline and noradrenaline is difficult when the

concentration of one amine exceeds that of the other (Häggendal, 1966; Callingham & Sharman, 1970). The catecholamine concentrations given in this study have not been corrected for their recoveries.

Experiments on isolated atria and on arterial blood pressure

The right atria were isolated and suspended in 60 ml of Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37°C. The spontaneous contractions were recorded with a force-displacement transducer on a Hellige Helcoscriptor. After 30 min of equilibration, noradrenaline or adrenaline was added every 3 min so that the final concentrations increased cumulatively from 5×10^{-9} to 5×10^{-5} M.

For measurement of arterial blood pressure chickens were anaesthetized with sodium pentobarbitone (200 mg/kg, i.m.). The body temperature was kept constant at 39.5-40.5°C by means of an electric pillow. The ischiadic artery and ischiadic vein were cannulated according to the method of Natoff & Lockett (1957). The arterial pressure was recorded on a Hellige compensation linear scriptor. Noradrenaline or adrenaline dissolved in saline was injected into the vein in volumes ranging from 0.65 to 1.1 ml.

Statistical analyses

Results are expressed as mean ± s.e. mean. The significance of the data was evaluated with Student's *t*-test. Regression equations and correlation coefficients (r) were calculated by the method of least squares using a computer programme. The number of experiments is n.

Results

Noradrenaline and adrenaline content of heart, spleen, brain, adrenal glands and blood plasma

Substantial amounts of both noradrenaline and adrenaline were found in all organs studied (Table 1). The adrenaline concentration expressed as percentage of noradrenaline plus adrenaline was highest in the adrenals (59%) and lowest in the spleen (16%). The relative adrenaline concentration of each organ was variable. However, there was no positive correlation when the concentrations in the hearts were compared with those of the spleens or brains from the same animal ($r < 0.36$). In 12 perfused hearts the mean noradrenaline concentration of the atria was 2.9 ± 0.4 fold higher than that of the ventricles. Essentially the same concentration ratio (2.6 ± 0.4 fold) was found for adrenaline (Table 1).

A total volume of approximately 30 ml blood could be obtained during exsanguination from a cannula located in the left ventricle. The mean concentrations of noradrenaline and adrenaline (Table 1) found when the total volume was removed in one step was 20 and 74 ng/ml, respectively, i.e. the adrenaline concentration was approximately four times higher than that of noradrenaline. In 14 animals the blood was collected in three consecutive 10 ml fractions. As shown in Table 2, the absolute concentrations of both amines increased considerably during exsanguination but the relative concentration of adrenaline remained constant. The concentration of adrenaline in the plasma was always significantly ($P < 0.001$) higher than it was in the adrenal glands.

Pretreatment of chickens with 6-hydroxy-

Table 1 The noradrenaline and adrenaline concentrations in various tissues of the adult chicken

Organ	n	*Organ weight (g) or plasma volume (ml)*	*Noradrenaline§ (NA)*	*Adrenaline (Ad)*	*Adrenaline (% of NA plus Ad)*
Heart*	21	4.2 ± 0.2	175 ± 19	83 ± 11 ng/g	33.1 ± 3.3
Spleen	67	2.2 ± 0.1	477 ± 27	90 ± 8 ng/g	16.4 ± 1.3
Brain	7	1.0 ± 0.02	588 ± 49	236 ± 70 ng/g	26.3 ± 5.1
Adrenal glands	28	0.08 ± 0.01	2.7 ± 0.2	4.0 ± 0.3 mg/g	58.9 ± 2.7
Plasma†	39	15.4 ± 1.0	20 ± 4	74 ± 11 ng/ml	79.5 ± 2.0
Whole heart‡	12	5.5 ± 0.3	177 ± 31	50 ± 11 ng/g	23.0 ± 4.2
Atria	12	0.5 ± 0.04	366 ± 36	102 ± 23 ng/g	21.3 ± 4.1
Ventricles	12	5.0 ± 0.3	158 ± 31	45 ± 10 ng/g	23.7 ± 4.3

* Non-perfused hearts. † Plasma was obtained by exsanguination through a cannula inserted into the left ventricle.
‡ Perfused hearts. § Units of concentrations as in the following column.

dopamine caused a decrease of both noradrenaline and adrenaline in the heart and the spleen. In the heart the noradrenaline concentration declined from 175 ± 19 ng/g (n = 21) to 35 ± 3 ng/g (n = 4) (20%) and the adrenaline concentration from 83 ± 11 ng/g to 19 ± 8 ng/g (23%). Similar changes were obtained in the spleen, where the concentrations were reduced to 78 ± 23 ng/g (16%) and to 26 ± 7 ng/g (29%) of the control values (Table 1), respectively.

Output of noradrenaline and adrenaline evoked from isolated hearts by sympathetic nerve stimulation, tyramine or 'high K^+-solution'

Hearts isolated from anaesthetized chickens were perfused at 34°C with a constant volume of 24-25 ml/minute. The rate of contraction was 129 ± 4 beats/min (n = 31). During a 30 min period the resting output of noradrenaline was 0.018 ± 0.009 ng g^{-1} min^{-1} while that of adrenaline was −0.004 ± 0.002 ng g^{-1} min^{-1} (n = 6). All estimations of the resting output were carried out *prior* to sympathetic nerve stimulation or to infusions of tyramine or of high K^+-solution.

Stimulation of the right sympathetic nerves of isolated chicken hearts at 20 Hz for 1 min (1 ms, 40 V) caused a mean maximum increase of 119 beats/minute. The perfusate was collected during sympathetic nerve stimulation and for 1.5 min thereafter. Ten min after the first stimulation period, the procedure was repeated and again the perfusate was collected for a total of 2.5 minutes. Both stimulation perfusates were pooled to give a total volume of 125 ml. It was found that sympathetic nerve stimulation evoked the output of 27 ± 5 ng/g noradrenaline (range 18 to 14) which represents about 15% of the total noradrenaline content of the heart. Moreover, sympathetic nerve stimulation caused an output of 7 ± 2 ng/g adrenaline (range 1.3 to 14.3) which represents 20 ± 5% of the sum of noradrenaline plus adrenaline and about 14% of the total adrenaline content of the heart (Table 3).

When tyramine (2.9×10^{-5} M) was infused for 30 min, the mean heart rate increased maximally from 111 to 210 beats/minute. The maximum increase was reached after 3-5 min of infusion and was followed by a gradual decline. The rate was still increased by 19 beats/min at the end of the tyramine infusion. During the 30 min exposure, tyramine caused a mean output of 48 ± 9 ng/g noradrenaline (range 18.6 to 145.6) and of 15 ± 2 ng/g adrenaline (range 2.6 to 39.9) which

Table 2 The noradrenaline and adrenaline concentration in the plasma during exsanguination

Plasma fractions	*Noradrenaline (NA) (ng/ml)*	*Adrenaline (Ad) (ng/ml)*	*Adrenaline (% of NA plus Ad)*
I + II + III	32 ± 8	100 ± 24	74 ± 2
I	16 ± 5	51 ± 14	77 ± 3
II	25 ± 6	99 ± 24	76 ± 3
III	51 ± 14	143 ± 37	71 ± 3

Chickens were exsanguinated by puncturing the left ventricle to remove 30-40 ml of blood, which was collected consecutively in 10 ml fractions I, II, and III. n = 14.

Table 3 The output of noradrenaline and adrenaline from the isolated heart of chicken in response to various stimuli and the remaining amine concentrations in the hearts

		Output of noradrenaline (NA) and adrenaline (Ad) into perfusate			*Concentration of NA and Ad remaining in the hearts†*		
*Stimulant**	n	*NA (ng/g)*	*Ad (ng/g)*	*Ad (% of NA plus Ad)*	*NA (ng/g)*	*Ad (ng/g)*	*Ad (% of NA plus Ad)*
Symp. nerve stim.	6	27 ± 5	7 ± 2	20 ± 5	157 ± 26	42 ± 10	22 ± 6
High K^+-solution	3	22 ± 2	9 ± 3	28 ± 8	123 ± 6	36 ± 16	22 ± 9
Tyramine	13	48 ± 9	15 ± 2	23 ± 4	160 ± 31	32 ± 5	19 ± 4

* The stimulants for noradrenaline and adrenaline output were sympathetic nerve stimulation (20 Hz, 2 x 1 min period), 'high K^+-solution' (108 mM K^+, 44 mM Na^+, 5 min infusion period) and tyramine (2.9×10^{-5} M, 30 min infusion period). ‡ The amine concentration was measured after the stimulation.

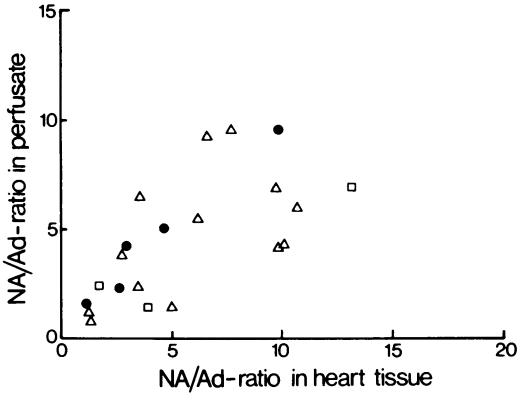

Figure 1 Isolated perfused heart of chicken. Variation of noradrenaline: adrenaline ratio (NA/Ad) in stimulation perfusate with NA/Ad ratio in heart tissue. Output of NA and Ad was in response to sympathetic nerve stimulation (20 Hz, 1 ms, 40 V; (●)), tyramine (2.9×10^{-5} M; (△)) or 'high K^+-solution' (K^+ 108, Na^+ 44 mM; (□)). Ordinate scale, output of NA and Ad into perfusate expressed as NA/Ad ratio. Abscissa scale, Na and Ad content remaining in the hearts after the end of perfusion expressed as NA/Ad ratio.

represents 23 ± 4% of the sum of both amines (Table 3). The relative adrenaline concentration remaining in these hearts was 19 ± 4% which is approximately the same as that of the perfusate.

In three hearts the K^+ and Na^+ concentrations of the Tyrode solution were changed from 2.7 to 108 mM $[K^+]_o$ and from 149.3 to 44 mM $[Na^+]_o$ for 5 minutes. This 'high K^+-solution' caused immediate arrest of the hearts and liberated noradrenaline and adrenaline in all experiments. The proportion of adrenaline released was not significantly different from that remaining in the hearts (Table 3).

A correlation between the individual rates of noradrenaline output and those of adrenaline output was not detected. However a plot of the noradrenaline: adrenaline ratios in perfusates of stimulated hearts against the ratios found in the corresponding heart tissue (Figure 1) revealed a positive correlation with a regression coefficient of 0.55 (correlation coefficient 0.78).

The effect of in-vivo *pretreatment with (−)-adrenaline on the amine content in heart, spleen, plasma, and cardiac perfusate*

(−)-Adrenaline (10 $\mu g\ kg^{-1}min^{-1}$) was infused into a brachial vein for 40 minutes. The spleen and the first 10 ml fraction of blood were removed for immediate estimation of noradrenaline and adrenaline, whereas the amines of the heart were measured after perfusion for 30 min with tyramine (2.9×10^{-5} M).

After infusion of adrenaline (Figure 2) the content of this amine was greatly elevated in heart

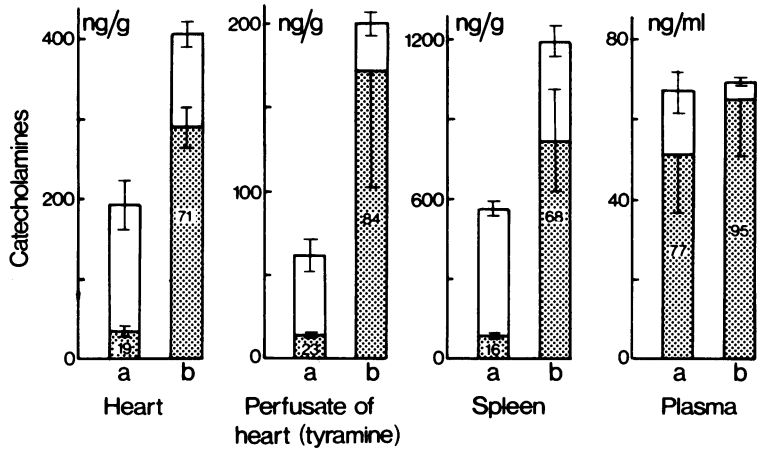

Figure 2 Effects of *in vivo* pretreatment with (−)-adrenaline on the concentrations of noradrenaline and adrenaline in heart, cardiac perfusate (during tryamine infusion), spleen, and blood plasma. Anaesthetized chickens received an infusion of 10 $\mu g\ kg^{-1}min^{-1}$ (−)-adrenaline for 40 minutes. Ordinate scale, noradrenaline (open bars) and adrenaline (stippled bars) concentrations in heart (after tyramine infusion), cardiac perfusate (during infusion of 2.9×10^{-5} M tyramine for 30 min), spleen, and blood plasma (1st 10 ml fraction during exsanguination, see methods sections and Table 2). Column (a) of each pair represent control values (no adrenaline pretreatment) and column (b) the values obtained after (−)-adrenaline pretreatment. The numbers within the bars indicate the % adrenaline concentration of the sum of both amines. The numbers of experiments for each column were 13, 3, 13, 3, 67, 3, 14, 3, (from left to right).

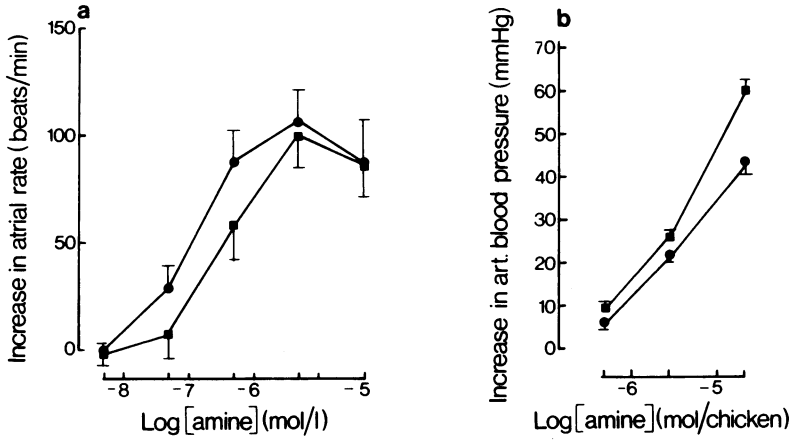

Figure 3 Comparison between cardiovascular potency of noradrenaline and adrenaline. (a) Cumulative concentration-response curves for noradrenaline (■) and adrenaline (●). Ordinate scale, increase in rate of contractions per min after exposure to various amine concentrations between 5×10^{-9} M and 5×10^{-5} M. Abscissa scale, molar concentrations of noradrenaline and adrenaline. (b) Dose-response curves for noradrenaline (■) and adrenaline (●). Ordinate scale, increase in arterial blood pressure (mmHg) after intravenous application of 0.5, 2.5 or 12.5 μmol per chicken. The weights of the chickens (1.0-1,2 kg) were almost the same. Each curve represents mean results from 6 atria or 6 anaesthetized chickens, respectively. Vertical bars indicate s.e. mean. For further details see methods and results sections.

and spleen, whereas the noradrenaline content decreased slightly. In the plasma significant changes in the concentration ratio of the two amines were not detected. Thus adrenaline now predominated over noradrenaline not only in the plasma, but also in the heart and the spleen. The relative concentration of adrenaline in the heart (71%) was nearly the same (84%) as that found in the heart perfusate during tyramine infusion (Figure 2).

The sum of the noradrenaline and adrenaline concentrations was doubled in the heart and the spleen after pretreatment with (−)-adrenaline, whereas the total output of both amines released by tyramine into the cardiac perfusate was increased 3.5-fold. As in the experiments without adrenaline pretreatment, a significant spontaneous release from the perfused heart could not be detected.

Cardio-vascular potency of noradrenaline compared with that of adrenaline in the chicken

The spontaneous contractions of right atria isolated from 12 chickens were recorded isometrically. The atria were suspended in 60 ml Tyrode solution at 35° C. After equilibration for 30 min, noradrenaline (6 atria) or adrenaline (6 atria) was added to the solution every 3 min so that cumulative concentration-response curves were obtained. As shown in Figure 3, both amines caused a dose-dependent increase in rate of contraction. The ED_{50} of noradrenaline (6×10^{-7} M) and the slope of the curve were identical with those of adrenaline.

The effects of noradrenaline and adrenaline on the isometric tension development were studied on electrically driven (3 Hz) left atria. It was found that the amplitude of contractions was increased by noradrenaline (ED_{50} 4.5×10^{-7} M) and by adrenaline (ED_{50} 6×10^{-7} M) to the same extent. Intravenous injections of either noradrenaline or adrenaline in increasing doses elevated the arterial blood pressure of chickens anaesthetized with sodium hexobarbitone. As shown in Figure 3, both amines were about equally effective in raising the arterial blood pressure.

Discussion

In the present study it has been found that adrenaline as well as noradrenaline is the sympathetic transmitter in the chicken. The evidence was as follows: 1. Sympathetic nerve stimulation, 'high K^+-solution' and tyramine caused the release of both amines from isolated perfused hearts (Table 3). 2. Chemical sympath-

ectomy by pretreatment with 6-hydroxydopamine reduced the concentrations of noradrenaline and adrenaline in heart and spleen. Degeneration of adrenergic neurones in the chicken heart following treatment with 6-hydroxydopamine was demonstrated by Bennett, Malmfors & Cobb (1973). 3. Both noradrenaline and adrenaline concentrations in the atria were two to three times higher than those in the ventricles. This corresponds with the relatively high overall density of the atrial adrenergic nerve plexus when compared with the ventricular innervation of the chicken heart (Bennett & Malmfors, 1970). 4. The increases of the arterial blood pressure and the stimulating effects on isolated atria evoked by noradrenaline and adrenaline showed nearly identical cardio-vascular potencies for both amines (Figure 3). This confirms the recent observation that the noradrenaline sensitivity of chicken left atria was not significantly different from the adrenaline sensitivity (Bennett & Malmfors, 1974). To our knowledge, a functional role for two transmitters in the peripheral sympathetic nervous system has not been described for any other species. In the frog the transmitter in the peripheral sympathetic nervous system is adrenaline, whereas in the mammal it is noradrenaline, but in neither of these classes do both transmitters function together as they appear to do in the chicken. Although relatively small amounts of adrenaline have been found in mammalian organs (see below), only noradrenaline was released from them. This holds true even when, for example, a maximally effective stimulant, such as dimethylphenyl-piperazinium iodide (DMPP) is applied to the rabbit isolated heart (Lindmar, Löffelholz & Muscholl, 1968). If, in this latter study, a release of adrenaline had been missed because of the limitations of estimation, it could have been only 1% of that of noradrenaline or less. There are striking differences between mammalian and frog hearts on the one hand (Holzbauer & Sharman, 1972) and chicken hearts on the other. Firstly, in the chicken heart both amines are present in relatively high concentrations so that there is twice as much noradrenaline as adrenaline. The predominance of noradrenaline over adrenaline has been previously reported (Ignarro & Shideman, 1968; Sturkie & Poorvin, 1973). Secondly, the sum of the absolute noradrenaline and adrenaline concentrations in the chicken heart is much lower than that of either mammalian and frog hearts.

The small adrenaline concentrations that have been detected in mammalian organs (Holzbauer & Sharman, 1972) were shown to originate from the circulation (Strömblad, 1961; Strömblad & Nickerson, 1961; Coleman & Glaviano, 1963; Muscholl, Kiefer & Lindmar, 1969). On the other hand, the phenylethanol-*N*-methyltransferase activity (PNMT) in the chicken heart was much higher than that in the rabbit heart (Lindmar & Wolf, 1975). Furthermore, Lindmar *et al.* (unpublished observations) have obtained some indirect evidence for a neuronal localization of this enzyme. It is, nevertheless, likely that part of the adrenaline stored in heart and spleen of the chicken has been removed from the circulation since the plasma levels of adrenaline were remarkably high (Table 2) and the nerves were able to concentrate (−)-adrenaline from the circulation (Figure 2). Moreover, the newly incorporated (−)-adrenaline was preferentially released by tyramine. On the other hand the possibility cannot be excluded that some of the catecholamines found in sympathetically innervated organs are located in non-neuronal catecholamine-storing structures which have been described in embryonic (Enemar, Falck & Häkanson, 1965) and mature chicken hearts (Bennett & Malmfors, 1970). However, the latter authors have pointed out that the number of round fluorescent cells in the heart of the adult chicken is relatively low when compared to mammalian hearts.

The nearly four-fold predominance of adrenaline over noradrenaline in the plasma was maintained throughout the gradual exsanguination although the noradrenaline and adrenaline concentrations in the plasma rose markedly, presumably because of an acute secretion of medullary hormones induced by hypovolemia. Lin & Sturkie (1968) also found about four times more adrenaline than noradrenaline in the plasma but the absolute amine concentrations were more than 10 times lower than those found in the present study. Although physiological plasma levels are unknown, it can be assumed that the values obtained at the beginning of exsanguination (adrenaline 51 ng/ml, noradrenaline 16 ng/ml, Table 2) were close to physiological amine concentrations. Thus, the chicken has a much higher plasma catecholamine concentration than mammals where the concentrations of noradrenaline and adrenaline are below 1 ng/ml (Holzbauer & Sharman, 1972). The high plasma levels of catecholamines suggest that, in the chicken, the medullary hormones play an important role in regulating cardiac performance and blood pressure as was previously assumed (Karg & Schams, 1966).

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