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The concentration of catecholamines in the brain of the domestic fowl *(Gallus domesticus)*

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Summary

1. The concentrations of adrenaline and noradrenaline in the brains of chickens (*Gallus domesticus*) have been determined using two different methods of fluorimetric analysis.
2. The concentrations of adrenaline, noradrenaline and also dopamine were found to increase with age, as did the relative amount of adrenaline present in the chicken brain.
3. It was observed that the differential assay of adrenaline by the trihydroxyindole method often underestimated the amount of adrenaline present.

Introduction

Studies on the catecholamines in the avian brain have indicated that adrenaline, noradrenaline and 3,4-dihydroxyphenylethylamine (dopamine) are present in this tissue. However, there is disagreement between authors using different methods of analysis about the presence of adrenaline in the brains of some avian species and also about the amount of this amine in the brain of the domestic fowl.

In the pigeon, Anton & Sayre (1964) found that adrenaline was present in the brain in a concentration that was approximately 8% of the noradrenaline concentration, and Juorio & Vogt (1967) reported adrenaline concentrations in parts of the pigeon brain which ranged from 14% to 33% of the corresponding noradrenaline concentrations. However, Aprison & Takahashi (1965) were unable to detect any adrenaline in the pigeon brain, a result which was also obtained by Falck, Ljunggren & Nordgren (1969). The latter authors were also unable to confirm the high concentration of adrenaline in the brain of the adult fowl which was observed by Juorio & Vogt (1967). In the course of a series of experiments in which the content of catecholamines in the chicken brain was being estimated using ion exchange resin chromatography followed by conversion of the catecholamines to fluorescent trihydroxyindole derivatives, a method comparable with those used by Aprison & Takahashi (1965) and by Falck *et al.* (1969), we observed that the estimates of the concentration of adrenaline were somewhat erratic and at times the method failed to detect the presence of any adrenaline. Since Falck *et al.* (1969) have criticized the use of bioassay procedures for the estimation of adrenaline in the avian brain, it was decided to re-examine this problem using a second chemical assay method that involved the separation of derivatives of adrenaline, noradrenaline and dopamine before their fluorimetric estimation.

Methods

Male " Ross 1 " chicks (Sterling Poultry Products Ltd.) were obtained at 1 day old and kept until required. The birds were killed by decapitation. The brains were rapidly removed, dissected on an ice cooled glass plate and either analysed immediately or kept at $-17°$ C for not longer than 1 day before analysis. Two procedures were used to estimate the catecholamines. In the first (the trihydroxyindole (THI) method; Iversen, 1963) the tissues were ground in 1% disodium ethylenediamine tetra-acetate solution and the proteins precipitated with 0·4 N perchloric acid. The extract was adjusted to pH 6·5 with 4 N potassium hydroxide and a pH meter, and after removal of the precipitate by centrifugation, passed through a column of Amberlite CG120 type 2 cation exchange resin (2·5 cm $\times$ 6 mm). The catecholamines were eluted with 10 ml of 1 N hydrochloric acid. Adrenaline and noradrenaline were oxidized with potassium ferricyanide and then treated with ascorbic acid and sodium hydroxide containing diaminoethane to form their respective lutines (von Euler & Lishajko, 1961). Adrenaline and noradrenaline were estimated by differential fluorimetry and in some cases the oxidation was carried out at pH 3·5 to obtain a second estimate of the concentration of adrenaline.

In the second procedure estimates of the concentrations of adrenaline, noradrenaline and dopamine were obtained by a modification of the method of Laverty & Sharman (1965). The tissues were homogenized in 0·1 N hydrochloric acid, containing ascorbic acid (10 mg/ml). The proteins were precipitated with perchloric acid (final concentration, 0·4 N) and the perchlorate removed by the addition of solid potassium chloride. After centrifugation, the amines in the clear supernatant were acetylated by the addition of acetic anhydride and solid sodium hydrogen carbonate. The acetyl derivatives were extracted, chromatographed on paper and estimated fluorimetrically as described by Laverty & Sharman (1965). Recovery of added noradrenaline and adrenaline by the THI method (mean $\pm$ S.E.M.) was $74 \pm 2\%$ ($n=6$) and $91 \pm 4\%$ ($n=6$) respectively. In the second method the recovery of added noradrenaline, adrenaline and dopamine was $55 \pm 3\%$ ($n=7$), $51 \pm 4\%$ ($n=7$) and $68 \pm 11\%$ ($n=7$). The observations are corrected for these recoveries. Each estimate was obtained from pooled tissues from two to twelve animals.

Analytical reagent grade chemicals were used throughout. Acetic anhydride and organic solvents were purified by triple distillation.

Results

Table 1 shows the concentrations of adrenaline and noradrenaline in the brains of chicks from 1 day to 8 weeks of age estimated by the THI method. The results at 8 weeks are comparable with those of Falck *et al.* (1969) who used birds of between 4 and 8 weeks of age. In some experiments using this method, however, it was not possible to detect the presence of adrenaline. Because of this, the method of Laverty & Sharman (1965) was used in which the acetyl derivatives of adrenaline, noradrenaline and dopamine are separated and estimated independently.

Table 2 gives the concentrations of the three amines estimated in the chick brain by this method and shows that both the absolute and relative concentration of adrenaline increases with the age of the birds.

Figure 1 illustrates the separation of the acetyl derivatives and distribution of the fluorescence derived from them after paper chromatography and also the fluorescence derived from an extract of diencephalic and mesencephalic tissue.

Discussion

The results presented confirm the observation of Gunne (1963) that adrenaline is present in the brain of the fowl and also show that the relative amount increases

TABLE 1. *Adrenaline and noradrenaline in fowl brain estimated by THI method*

Age	Brain region	Adrenaline (μg/g)	Noradrenaline (μg/g)	% methylated
1 week	Cerebral hemispheres	<0·02	0·20	<10
	Diencephalon plus mesencephalon	0·09	0·53	14·5
	Rhombencephalon*	0·06	0·29	17·1
2 weeks	Cerebral hemispheres	<0·02	0·16	<13
	Diencephalon plus mesencephalon	0·13	0·29	31
	Rhombencephalon*	<0·02	0·37	<6
3 weeks	Cerebral hemispheres	<0·02	0·20	<10
	Diencephalon plus mesencephalon	0·13	0·63	17·1
	Rhombencephalon*	<0·02	0·58	<4
8 weeks	Cerebral hemispheres	0·04±0·01	0·16±0·01	20
	Diencephalon plus mesencephalon	0·14±0·04	0·56±0·04	20
	Rhombencephalon*	0·19±0·06	0·20±0·03	48·7

* Rhombencephalon comprises cerebellum, pons and medulla.

TABLE 2. *Concentration of adrenaline, noradrenaline and dopamine in fowl brain estimated by the acetylation method*

Age	Brain region	Dopamine (μg/g)	Adrenaline (μg/g)	Noradrenaline (μg/g)	% methylated
2 days	Cerebral hemispheres	0·115	0·006	0·131	4·4
	Diencephalon plus mesencephalon	0·038	0·013	0·416	3·0
	Rhombencephalon*	<0·002	<0·002	0·182	<1·1
8 days	Cerebral hemispheres	0·354	0·006	0·218	2·7
	Diencephalon plus mesencephalon	0·094	0·053	0·600	8·1
	Rhombencephalon*	0·016	0·026	0·345	7·0
3 days	Cerebral hemispheres	0·224	0·010	0·133	7·0
	Optic lobes	0·007	0·010	0·303	3·2
	Diencephalon plus mesencephalon minus optic lobes	0·146	0·068	0·678	9·1
	Cerebellum	0·002	<0·002	0·198	<1·1
	Medulla	0·056	0·029	0·576	4·8
7 days	Hypothalamus	0·283; 0·313	0·145; 0·162	0·830; 0·890	15·2
6 months	Cerebral hemispheres	0·468; 0·416	0·025; 0·014	0·265; 0·285	6·8
	Optic lobes	0·010; 0·004	0·035; 0·016	0·400; 0·306	6·6
	Diencephalon plus mesencephalon minus optic lobes	0·120; 0·086; 0·103	0·400; 0·400; 0·180	1·362; 1·014; 0·670	24·0
	Cerebellum	0·004; 0·006	<0·002; 0·004	0·268; 0·202	0·8
	Medulla	0·023; 0·023	0·138; 0·070	0·474; 0·284	21·0

* Rhombencephalon comprises cerebellum, pons and medulla.

with age. The concentration of dopamine and noradrenaline was also found to increase with age.

It is known that the differential estimation of adrenaline and noradrenaline is difficult when the concentration of one amine greatly exceeds that of the other (Häggendal, 1966). During the present experiments it was found that although adrenaline was clearly demonstrable by the acetylation procedure, the THI method often underestimated the amount present or failed to detect any at all. Juorio & Vogt (1970) using bioassay have obtained estimates for the concentration of adrenaline in the chick brain which are comparable with those obtained here by the acetylation method and they have also confirmed their earlier observations on the pigeon and chicken brain.

It can only be concluded that differential assay by the THI procedure should not be used as the only method for obtaining evidence concerning the presence or absence of small amounts of either adrenaline or noradrenaline when the other amine is present in quantity.

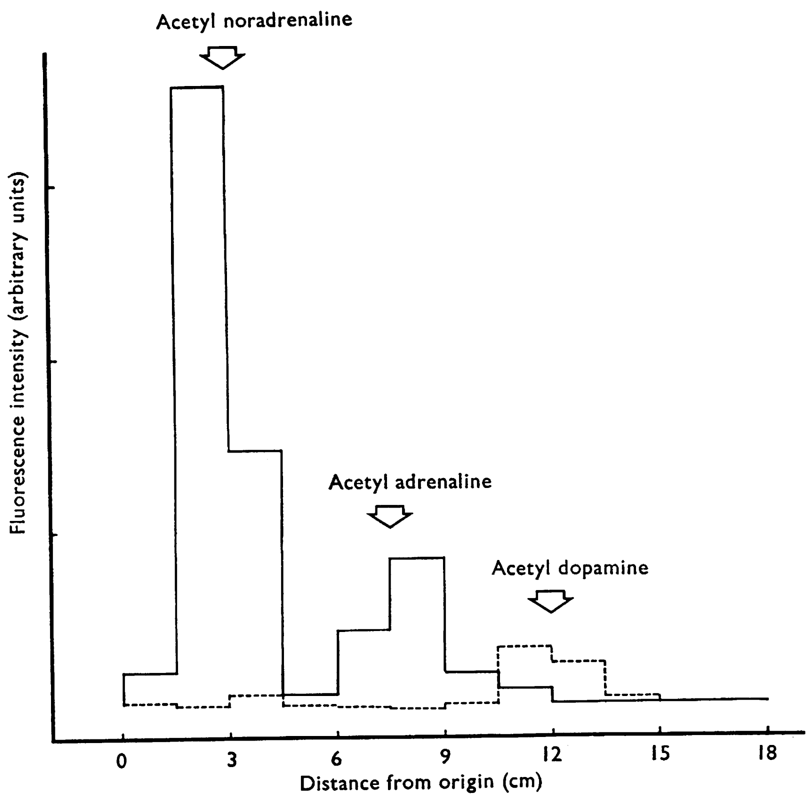

FIG. 1. Separation of the acetyl derivatives of the catecholamines in the diencephalon and mesencephalon of adult fowls on a paper chromatogram following extraction, acetylation and condensation with 1,2-diamino ethane. ——, Values derived from fluorescence with characteristics of that from adrenaline and noradrenaline; - - - -, values derived from fluorescence with characteristics of that from dopamine.

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