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The effect of age and thyroid hormones upon the ability of the chick heart to deaminate monoamines

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The effect of age and thyroid hormones upon the ability of chick heart homogenates to metabolize monoamines has been investigated. 5-Hydroxytryptamine is entirely metabolized by a monoamine oxidase (MAO) with the characteristics of MAO-A, whereas some of the tyramine and all of the benzylamine are oxidatively deaminated by a clorgyline-resistant, but semicarbazide-sensitive enzyme, with a similar subcellular distribution to that of MAO. The remainder of the tyramine deamination is brought about by MAO-A and MAO-B. The specific activities of both clorgyline-sensitive and resistant enzymes are increased by the same proportion by increase in age or by treatment with (−)-thyroxine, and decreased by 2-thiouracil. The significance of these results is discussed.

The deamination of monoamines in animal tissues is brought about mainly by the enzyme monoamine oxidase (MAO; EC 1.4.3.4), which has been shown to exist in at least two forms, designated MAO-A and MAO-B, according to their relative sensitivities to the selective irreversible inhibitor, clorgyline (Johnston, 1968). The enzyme is localized largely in the mitochondrial outer membrane (for reviews, see Greenawalt, 1972; Blaschko, 1974; Tipton, 1975). However, other deaminating activities have been found. For example, in the rat heart, benzylamine is also metabolized by a clorgyline-resistant, semicarbazide-sensitive enzyme occurring mainly in the microsomes and cytoplasm (Lyles & Callingham, 1975).

There is now considerable evidence that growth and development influence the MAO activity (for review, see Gripois, 1975), as do changes in the circulating concentrations of hormones such as thyroid (Novick, 1961) and glucocorticoids (Avakian & Callingham, 1968). However, experiments on rats have provided most of the available evidence although species differences may occur. The developing chick was used by Ignarro & Shideman (1968), who found that the specific activity of the MAO in the heart changed with age, both before and after hatching, when tryptamine was used as substrate. From the third day of incubation until two days before hatching, the MAO activity rose, only to fall at hatching before increasing again between 10 and 15 days later.

In this present study, we have attempted to discover if any clorgyline-resistant activity is present in the heart of the chick after hatching, and to see if this activity is affected by age and thyroid hormones in a similar manner to the clorgyline-sensitive MAO.

Some of this work has appeared in preliminary form (Callingham & Fowler, 1977).

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MATERIALS AND METHODS

The radioactive substrates for MAO [^{3}H]tyramine, [^{3}H]5-hydroxytryptamine and [^{14}C]benzylamine were obtained from New England Nuclear GMBH, Dreieichenhain, Germany; The Radiochemical Centre, Amersham, U.K.; and ICN Pharmaceuticals, Hersham, U.K. respectively.

Clorgyline hydrochloride was a gift from May & Baker Ltd., Dagenham, U.K. Semicarbazide hydrochloride, (−)-thyroxine (free acid) and 2-thiouracil were obtained from Sigma London, Kingston-upon-Thames, U.K. All other materials used were standard laboratory reagents of analytical grade where possible.

Male chicks were obtained newly hatched from SAPPA Ltd., Bury St. Edmunds (Comet strain), and from Ross Poultry Ltd., Woodhall Spa (Ross mixed strain).

Chicks were decapitated and their hearts removed. After trimming off fat and large blood vessels, the hearts were blotted and homogenized in 1mM potassium phosphate buffer, pH 7·8 in a conical glass homogenizer. The crude homogenates were centrifuged at 600 *g* for 15 min to remove nuclei and cell debris. The resulting supernatants were used for assay of their MAO activity.

Chicks were made hypothyroid by the addition of 2-thiouracil (0·2% w/w) in the feed beginning on the second day after hatching. Their corresponding age-matched controls received the same feed without drug.

Chicks were made hyperthyroid by daily injections of (−)-thyroxine (1 mg kg^{-1}, s.c.) for 11 days beginning on the second day after hatching. They were killed 24 h after the last injection. Their corresponding age-matched controls received equivalent doses of 0·9% saline solution.

Subcellular distribution

Twelve chicks aged 32 days were killed and the hearts dissected as before. The hearts were then pooled into 4 groups and homogenized in a Sorvall Omnimixer in 0·154 M potassium phosphate buffer, pH 7·8. After removal of nuclei and debris, the resulting low-speed supernatants were centrifuged at 6500 *g* for 20 min to produce crude mitochondrial fractions. The pellets were washed twice with buffer and finally resuspended to produce the mitochondrial fractions which were used in the subsequent experiments. The supernatants from the crude mitochondrial fractions were centrifuged at 15 000 *g* for 15 min to remove any remaining broken mitochondria. The resulting supernatants were then centrifuged at 70 000 *g* for 120 min to produce high speed supernatants containing soluble or cytoplasmic enzymes, and microsomal pellets that were washed once. All appropriate volumes were measured.

Monoamine oxidation was assayed radiochemically by the method of McCaman, McCaman & others (1965) as modified by Callingham & Laverty (1973) with [^{3}H]tyramine, [^{3}H]5-hydroxytryptamine (^{3}H-5-HT) and [^{14}C]benzylamine as substrates. In experiments with clorgyline and semicarbazide, the homogenates were pre-incubated with the inhibitors for 20 min before the addition of substrate. Specific activities were expressed in nmol (of substrate metabolized) (mg protein)$^{-1}$ h^{-1}, calculated as means ± s.e.m.

Protein contents were measured by the microbiuret method of Goa (1953), with bovine serum albumin as standard.

Statistical significance between groups was determined by Student's *t*-test on the absolute values.

RESULTS

Composition and subcellular distribution of the deaminating activities

The effects of clorgyline upon the ability of chick heart homogenates to deaminate 5-HT, tyramine and benzylamine are shown in Fig. 1.

The metabolism of 5-HT was inhibited by increasing concentrations of clorgyline in a single sigmoid manner, which corresponds to an action of MAO-A alone. Three components were seen when tyramine was used as substrate. This would indicate that most of the activity is MAO-A, with a small proportion of activity that is less sensitive to clorgyline but is inhibited by high concentrations, i.e., MAO-B, and a residual activity that was unaffected by clorgyline at concentrations up to at least 10^{-3}M. Benzylamine metabolism in contrast, was, almost completely resistant to clorgyline inhibition.

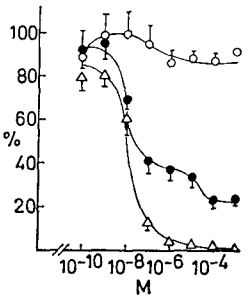

FIG. 1. The effect of clorgyline (M) upon the *in vitro* deaminating activity (%) of the hearts of chicks aged between 6 and 40 days. Each curve is derived from the means and standard errors of the ratios from groups of four chicks, all assays being performed in duplicate with 1 mM substrate. The curves were not significantly altered over the age range of the chicks. ○—○, benzylamine; ●—●, tyramine; △—△, 5-HT.

The deamination of 5-HT was not inhibited by 10^{-3}M semicarbazide, whereas 25% and over 90% of tyramine and benzylamine metabolism were inhibited respectively. The inhibition of activity by clorgyline and semicarbazide was additive. These results are summarized in Table 1.

Table 1. *Effect of clorgyline* (*I*, 10^{-3}M), *semicarbazide* (*II*, 10^{-3}M), *and both drugs upon the deamination of 5-HT, tyramine and benzylamine in the chick heart.* All assays were in duplicate with 1 mM substrate concentrations and four pairs of 32 day old chicks, and the results expressed in terms of the means ± standard errors of the ratios of the percentage inhibition of the deamination caused by each drug.

	% Inhibition of deamination		
Substrate	I	II	I + II
5-HT	97·1 ± 1·4	0 ± 8·1	98·2 ± 0·6
Tyramine	88·9 ± 2·9	25·7 ± 9·6	96·1 ± 2·0
Benzylamine	0 ± 2·3	91·0 ± 4·1	99·0 ± 1·0

A closely similar subcellular distribution of the activities for all three substrates was found (Table 2). In each case the bulk of the activity was seen in the mitochondrial fraction. There was significantly more

Table 2. *Subcellular distribution of the 5-HT, tyramine and benzylamine metabolizing activities in the chick heart.* Values are expressed as percentage of activities in each subfraction/sum of activities in the subfractions, and expressed in terms of means ± standard errors of the ratios. All assays were performed in duplicate with 1 mM substrate and four groups of three 32 day old chicks. Significance levels were determined by 95% confidence limits (Goldstein, 1967).

Substrate	% of total activity Mitochondria	Microsomes	Supernatant
5-HT	86·5 ± 2·8	9·2 ± 2·5	4·3 ± 1·7
Tyramine	86·7 ± 2·2	9·6 ± 1·1	3·7 ± 1·1
Benzylamine	80·0 ± 2·9*	17·6 ± 2·5**	2·4 ± 0·6

* = significantly different from tyramine
** = significantly different from tyramine and 5-HT.

microsomal activity towards benzylamine than towards the other substrates, but it comprised only 17·6% of the total activity. Negligible activity was found in the high speed supernatant fractions.

Effects of age

The chicks were killed at 6, 21 and 40 days after hatching. Although there were increases in heart and body weights at 21 and 40 days, no change in the specific activities of the MAO towards either tyramine or benzylamine were seen until 40 days, when the activities had increased to 156 and 160% respectively of the values at 6 days (Table 3). The shapes of the clorgyline-inhibition curves for both substrates at all three ages were not significantly different from each other.

Table 3. *Effect of age upon the body weight, heart weight, and tyramine and benzylamine deaminating activities of the chick heart.* All assays were performed in duplicate with 1 mM substrate concentrations. Results are expressed in terms of the means ± standard errors of the means for absolute activities of groups of four chicks aged 6, 21 and 40 days. Bracketed figures show the percentage change from values determined in the 6 day old chicks. Significance levels were determined by Student's *t*-test on the absolute values.

Age (days)	Body wt (g)	Heart wt (mg)	Specific activities (nmol (mg protein)$^{-1}$ h^{-1}) Tyramine		Benzylamine	
6	60	270	47·1 ± 4·8	(100)	28·2 ± 2·1	(100)
21	137	829	50·3 ± 5·9	(107)	30·6 ± 1·8	(109)
40	341	2155	73·5 ± 3·8**	(156)	45·0 ± 3·0**	(160)

** $P < 0.01$.

Effects of thyroid hormone

When chicks were fed with thiouracil (0·2% w/w), there was a significant decrease in their body weights to 72% of their corresponding controls. A reduction in the weight of their hearts to 58%, accompanied by increased deposition of fat on the outer surface, also occurred (Table 4). The specific activities of the deaminating enzymes for tyramine and benzylamine were 73 and 56% of their corresponding control values respectively. Although only the fall in benzylamine metabolism was significant, the Vmax values for tyramine in the hypothyroid hearts were significantly reduced to 48% of control without any change in the Km of the enzyme (Table 4).

Chicks were made hyperthyroid by daily injections of (−)-thyroxine (1 mg kg^{-1}) for 11 days. By this time the body weight of the chicks had fallen to 80% of control without any significant change in heart weight. Specific activities of the deaminating enzymes for tyramine and benzylamine were significantly increased to 145 and 149% of their corresponding control values respectively.

DISCUSSION

From the results presented here, the oxidative deamination of monoamines in chick heart homogenates is brought about by the action of more than one enzyme. By the use of the selective inhibitor, clorgyline, and the convention introduced by Johnston (1968) of designating the more sensitive enzyme activity as MAO-A and the less sensitive as MAO-B, 5-HT metabolism in the chick heart is brought about by MAO-A alone. This has been found to be the case in several other tissues, including rat brain (Johnston, 1968), rat heart (Lyles & Callingham, 1975) and the human heart (Parkinson, D. & Callingham, B. A. unpublished). Tyramine is metabolized by MAO-A, MAO-B and a clorgyline-resistant but semicarbazide-sensitive enzyme, while benzylamine is metabolized by this last enzyme alone.

The clorgyline-resistant enzyme found in the chick heart homogenates differs in its subcellular localization from that in seen in some other tissues, in that it is largely in the mitochondrial fraction. In the rat heart, for example, the clorgyline-resistant activity is mainly in the microsomes and cytoplasm (Lyles & Callingham, 1975). Blood vessels also contain an amine oxidizing activity (Thompson & Tickner, 1951; Dyer & Weber, 1971), which, in the rat aorta,

Table 4. *Effect of 2-thiouracil (hypothyroid) and (−)-thyroxine (hyperthyroid) upon the body weight, heart weight, and tyramine and benzylamine deaminating activities of the chick heart.* All assays were determined in duplicate with substrate concentrations of 1mM, and the results expressed in terms of means ± standard errors of the mean for each group of four chicks. Km and Vmax determinations for the tyramine deamination for 40 day old control and hypothyroid chicks were calculated by computer program from determinations made at four time intervals (5, 10, 20 and 30 min) with four substrate concentrations (0·125, 0·25, 0·5 and 1mM tyramine). Bracketed figures show the values as percentages of their respective controls. Statistical significance between groups was determined by Student's *t*-test on the absolute values.

				Specific activities (nmol (mg protein)$^{-1}$ h^{-1})	
Status	Age (days)	Body wt (g)	Heart wt (mg)	Tyramine	Benzylamine
Control	40	341 ± 17 (100)	2155 ± 94 (100)	†73·5 ± 3·8 (100)	45·0 ± 3·0 (100)
Hypothyroid	40	247 ± 11** (72)	1250 ± 110** (58)	††54·0 ± 10·1 (73)	25·3 ± 5·3* (56)
Control	13	124 ± 5 (100)	794 ± 54 (100)	20·9 ± 1·6 (100)	15·8 ± 1·2 (100)
Hyperthyroid	13	99 ± 5* (80)	782 ± 37 (98)	30·3 ± 2·7* (145)	23·5 ± 1·9* (149)

† Km = 100 μM, Vmax = 96·0 ± 6·8 (100).
†† Km = 123 μM, Vmax = 46·2 ± 9·3** (48).
** $P < 0·01$ * $P < 0·05$.

is to be found in the high-speed supernatant fraction (Coquil, Goridis & others, 1973). Although, with the evidence presently available it is not possible to be certain, this clorgyline-resistant activity in the homogenates of the chick heart must come predominantly from the cells of the myocardium. A contribution from neurons, connective tissue and blood vessels cannot be ignored, but they would be very active indeed if none of the enzyme were to be found in the cardiac muscle cells. In the rat at least, treatment with 6-hydroxydopamine does not reduce MAO activity sufficiently to show that the neuronal enzyme contributes significantly to the activity of crude homogenates (Horita & Lowe, 1972). It is clear, however, that a great deal more needs to be done to resolve the uncertainty concerning not only the localization, but also the nature and function of this clorgyline-resistant enzyme. It is possible that it is similar to the benzylamine oxidase found, for example, in pig plasma (Buffoni, Della Corte & Hope, 1977) or to the lysyl oxidase involved in the formation of collagen and elastic fibres (see Blaschko, 1974). Unfortunately, the problem concerning the possible function of MAO in heart muscles is far from resolved. In the chick heart, both MAO and the clorgyline-resistant activity are associated with the mitochondria. If they are not located in different tissues in the heart they may well have a very similar but, at present, elusive part in the metabolism of the myocardium.

The increase in the specific activity of the MAO in the heart of the chick, with tyramine as substrate, that occurs with age after hatching, confirms the observations of Ignarro & Shideman (1968), and resembles that previously seen in the rat heart (Horita, 1967). However, the increase in the clorgyline-resistant benzylamine metabolizing activity, to the same extent and with the same time course as that seen with tyramine as substrate, does not occur in the rat heart, where little or no increase is found as the rat grows older (Callingham & Lyles, 1975).

This parallel increase in the clorgyline-sensitive and clorgyline-resistant enzymes is also seen when the chicks are made hyperthyroid by daily injections of thyroxine. On the other hand, hypothyroidism leads to a significant decrease in the clorgyline-resistant specific activity. Although in the present study the decrease in specific activity for MAO was not significant, the Vmax value for MAO from the hypothyroid chicks was significantly reduced without any change in Km (Table 4). Thus whatever the nature of the clorgyline-resistant enzyme, it responds in the same way as MAO in the chick heart to age and to changes in thyroid status. However, in the rat heart, while the MAO responds in a similar way to changes in thyroid status, the clorgyline-resistant enzyme is much less sensitive (Lyles & Callingham, 1974). This difference may reflect the different subcellular localizations of the two species, especially since Okamoto (1971) has shown that the response to thyroxine of enzymes located in the outer membrane of the rat liver mitochondrion is in the opposite direction to the responses of those enzymes on the inner membrane.

The mechanism by which age and thyroid status exert their effects in the chick are as yet unknown. However, in the rat, there is a decrease in the apparent rate of degradation of the MAO with age (Della Corte & Callingham, 1977), whereas thyroxine appears to exert its effect either by increasing the rate of synthesis of the enzyme or by an effect on the structure of the mitochondrial membrane, to activate the enzyme by an allosteric process, (Lyles & Callingham, 1974). Houslay & Tipton (1973) have suggested that MAO is allosterically regulated by lipids and thyroidectomy in rats has been shown to lead to an increase in the degree of lipid unsaturation in the liver mitochondrial membrane, while thyroxine decreases this unsaturation (Hulbert, Augee & Raison, 1976). This could imply that the effects on the MAO and chick clorgyline-resistant enzyme are due to the location of the enzymes in the mitochondrion. Changes in the structure of the mitochondrial membrane lipids could then affect both enzymes to the same degree.

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