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The Donor Activity for Peroxidase and the Effect on the Thyroid Gland of Certain Tyrosine Derivatives

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Tyrosine and some tyrosine derivatives have been tested as donors for myeloperoxidase by an amperometric technique. Some of the more active compounds (tyrosine ethylester, tyrosine-pentylester and dimethyltyramine) were also tested for thyrostatic action in rats, but were found to be inactive in this respect.

It has been suggested that a group of antithyroid agents, which inhibit the biosynthesis of thyroxine formation, are effective because they are donors for peroxidase [1-2]. This enzyme participates in the biosynthesis of the thyroid hormone(s) [3-6] by forming iodinated tyrosine derivatives from tyrosine, iodide, and hydrogen peroxide. In the presence of antithyroid agents less hydrogen peroxide would be available for the iodination of tyrosine and less thyroid hormone would be formed. In search for potential antithyroid agents the authors have tested compounds related to tyrosine as donors for peroxidase. The most active compounds were then tested *in vivo* for antithyroid action in rats.

EXPERIMENTAL

Materials. The following commercial products were used: tyrosine-methylester hydrochloride, tyrosine ethylester hydrochloride, N-acetyltyrosine, N-acetyltyrosine ethylester (all three from Mann Research Lab.), tyramine hydrochloride (Hoffmann-La Roche), and N,N-dimethyl tyramine (hordenine) sulfate (Theodor Schuchardt GmbH & Co.). Propyl- to heptyltyrosine ester hydrochlorides were prepared by heating tyrosine with an excess of the corresponding alcohol in the presence of dry hydrogen chloride [7-8]. The products were precipitated by diethyl ether and recrystallized twice from acetone-ether.

The elemental composition of the synthesized compounds was determined to be as follows:

$C_{12}H_{18}ClNO_3$. Theory: Cl 13.65, N 5.39. Found: Cl 13.70, N 5.39.
$C_{13}H_{20}ClNO_3$. Theory: Cl 12.95, N 5.12. Found: Cl 12.94, N 5.04.
$C_{14}H_{22}ClNO_3$. Theory: Cl 12.32, N 4.87. Found: Cl 12.38, N 4.73.
$C_{15}H_{24}ClNO_3$. Theory: Cl 11.75, N 4.64. Found: Cl 11.69, N 4.64.
$C_{16}H_{26}ClNO_3$. Theory: Cl 11.23, N 4.43. Found: Cl 11.28, N 4.36.

N-Methyl tyrosine was synthesized according to Corti [9]. *p*-Hydroxybenzylamine, N,N-diethyl-*p*-hydroxybenzylamine, and *β,β*-dimethyltyrosine [10] (neotyrosine) were obtained from Dr. Å. Jönsson and myeloperoxidase [11] with a relative purity of 0.5—1.0 was a generous gift from Dr. K. Agner.

Methods. In preliminary experiments, the reaction between the tyrosine derivatives and hydrogen peroxide in the presence of peroxidase was followed spectrophotometrically at 250 mμ, where the reaction products absorbed light strongly in comparison with the substrates. However, since the chemical structure and molar absorbancy of the reaction products were unknown [12] this method did not allow a quantitative comparison between the rates of utilization of the different substrates. A method was then developed in which the reaction was followed by amperometric determination of remaining hydrogen peroxide with a rotating platinum electrode. The latter consisted of a 0.5 mm platinum wire sealed into a glass tube with the exposed part about 5 mm long. It was rotated at 800 rpm and polarized at −0.6 V *versus* a saturated calomel electrode. The current was read with a galvanometer with a maximum sensitivity of 1.75×10^{-9} A/mm. After a sufficiently stable current was obtained the reaction was started by addition of the enzyme to the test system (if necessary, corrections for small spontaneous decreases were made). Readings were then taken every tenth second for 1.0 to 1.5 min. It was found that as long as less than 75 % of the hydrogen peroxide has been consumed the reaction was of first order with respect to hydrogen peroxide under the reaction conditions studied and the apparent first order velocity constant was a linear function of the enzyme concentration. The initial hydrogen peroxide concentration (1×10^{-4} M) was chosen as a compromise between sensitivity of the measuring device and the inactivating effect of hydrogen peroxide on myeloperoxidase [13]. The low initial concentration of tyrosine derivatives (1×10^{-4} M) was used as certain of the compounds studied had a very limited solubility. (Experiments with dimethyltyramine showed that the enzyme was not saturated with substrate at this concentration). The higher members of the *n*-alkylesters of tyrosine gave very insoluble reaction products, which apparently adhered to the electrode surface and decreased the sensitivity of the electrode (shown as a downward deviation from the semi-logarithmic plot of the reaction curve at longer time intervals). In these cases the electrode was cleaned between each experiment by immersion in ethanol for a few minutes.

Iodine was determined in rat thyroid homogenates by an adaption of the method of Skanse and Hedenskog [14] and ^{131}I activity was measured in a well scintillation counter by a standard technique.

RESULTS

The ability of tyrosine and its carboxyl esters to function as donors for myeloperoxidase was investigated and the results are given in Table 1. It is evident that the esters are more active than tyrosine itself and that the activity increases with increasing length of the alkyl chain with a maximum for the

Table 1. Tyrosine esters as donors for peroxidase. The test system contained 10^{-4} M tyrosine or its ester, 10^{-4} M hydrogen peroxide, 0.05 M phosphate buffer pH 7.4, 0.01 M KCl, and 76—304 μg myeloperoxidase in a final volume of 30 ml at 30°C. The activity is given as the apparent first order velocity constant corresponding to 1 mg peroxidase in the test system.

Compound	*Activity* min^{-1} mg^{-1}
Tyrosine	1.45
Tyrosine-methyl ester	4.79
» ethyl »	5.72
» propyl »	8.50
» butyl »	9.35
» pentyl »	9.15
» hexyl »	9.80
» heptyl »	7.90

Table 2. Tyrosine and tyramine derivatives as donors for peroxidase. Reaction conditions were as in Table 1.

Compound	*Activity* min^{-1} mg^{-1}
Tyrosine	1.04
N-Methyltyrosine	1.58
N-Acetyltyrosine	0.76
N-Acetyltyrosineethylester	5.85
β,β-Dimethyltyrosine	0.42
Tyramine	17.3
N-Dimethyltyramine	18.4
3,5-Diiodotyrosine	1.08

butyl- through hexyl- compounds. The ethyl- and pentylesters were also assayed for thyrostatic activity at the Research Laboratories of AB ASTRA, Södertälje, by Dr. A. Åström. Groups of five rats were injected daily for 27 days, and it was found that neither of these compounds (dose 0.1 g/kg body weight) had any effect on the size, histological picture, or ^{131}I uptake of the thyroid (Åström, A. and Walde, N., unpublished).

Some other derivatives of tyrosine were assayed next for peroxidase activity (Table 2). Tyramine and its dimethyl derivative were the most active of these compounds, but the effect of tyramine on the thyroid gland was not investigated since this compound has a strong vasopressor activity. However, dimethyltyramine was tested at the Research Laboratories of AB ASTRA by the same technique used for the tyrosine esters and it was found that a single injection (dose 0.05 g per kg body weight) of the compound depressed ^{131}I uptake in the thyroid gland after 4 h. This effect was less than that obtained after the same dose of propylthiouracil and disappeared after 17 h. Nevertheless, these preliminary experiments indicated that dimethyltyramine had an inhibitory effect on thyroid function and it was decided to study the effect of prolonged administration of the compound on the thyroid function of rats. The results obtained (Table 3) did not show any effect of the treatment on either thyroid weight, iodine content, or ^{131}I uptake.

Table 3. Effect of dimethyltyramine on thyroid function. Each group consisted of 5 rats of 257 ± 6 g average body weight. One group received daily intraperitoneal injections of 0.05 g dimethyl-tyramine sulfate/kg body weight for 25 days, whereas the other group received no treatment. At the end of the experimental period each rat received 25 μC ^{131}I by intraperitoneal injection. The rats were killed 24 h later; the thyroids were excised, weighed, and homogenized in deionized water and the radioactivity and total iodine content determined. Values given are means ± standard error.

Treatment	*Thyroid weight* mg	*Iodine content* μg/mg fresh weight	^{131}I *uptake* %
Control	28 ± 4	0.19 ± 0.017	54 ± 6
Dimethyltyramine	22 ± 4	0.22 ± 0.036	65 ± 16

DISCUSSION

The data obtained in this investigation show that if tyrosine is esterified or decarboxylated, its donor action in the peroxidase reaction increases. This suggests that a negative charge on the donor decreases the efficiency of the latter, but more kinetic data are necessary in order to allow a detailed interpretation of these effects. However, neither the tyrosine esters nor dimethyltyramine were active antithyroid agents — although they were active donors for peroxidase. This observation appears to invalidate the aforementioned theory that antithyroid agents may suppress the biosynthesis of thyroid hormone(s) by competition for hydrogen peroxide, but it is possible that the compounds studied in this investigation did not enter the thyroid gland due to permeability effects. Another possibility would be that these compounds were rapidly metabolized in other parts of the body. The occurrence in different tissues of esterases which split tyrosine esters has in fact been reported [15] and oxidative degradations of dimethyltyramine by tissue preparations [16,17] and by the intact animal (dog) [18] have also been described. In this connection it may also be questioned if results obtained with a peroxidase from white blood cells are applicable to the peroxidase occurring in the thyroid gland. As Klebanoff *et al.*[6] have shown that the thyroid peroxidase may be effectively replaced by myeloperoxidase in their test system this objection seems not to be valid.

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