INHIBITION OF CATECHOL-O-METHYLTRANSFERASE BY HYDROXYBENZENES AND RELATED COMPOUNDS

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The inhibitory action of 1, 2, 3-trihydroxybenzene (pyrogallol) on the activity of catechol-O-methyltransferase (COMT) is well known (1-4). Recently, 1, 3, 5-trihydroxybenzene (phloroglucinol) and 2, 4, 6-trihydroxy-1-propionophenone (THPP) have been shown to exert spasmolytic action in experimental animals and to relieve spastic disorders of the urinary tract and biliary ducts in patients (5-10). Comparing the relative effects of trihydroxybenzenes on the smooth muscle organs, Inoue and associates (11-13) have found that these compounds possess catecholamine-like actions and potentiate the effect of catecholamines. Therefore, it is expected that phloroglucinol and THPP also inhibit the COMT activity. In the present experiments, the effects of phenol, phenolic ethanolamines, dihydroxybenzenes, trihydroxybenzenes and related compounds on the COMT activity of rat liver were studied *in vitro*.

METHODS

1. Preparation of COMT from rat liver

Male Wistar rats weighing 200 to 250 g were fed on the commercial diet and given water *ad libitum* for one week. The animals were killed by head amputation, exsanguinated, and the livers were immediately removed and chilled. Livers were homogenized in 4 volumes of isotonic KCl and centrifuged at $100,000 \times g$ for 1 hour. The soluble supernatant was stocked in a refrigerator at -18°C until used as crude COMT preparation.

2. S-Adenosylmethionine as a methyl donor

S-Adenosylmethionine was isolated from yeast cultures which were incubated with methionine following the method of Steckel *et al.* (14). The S-Adenosylmethionine was 5 to 10 times more active than the commercial product as a cofactor for the COMT. It was stored *in vacuo* until used.

3. Assay of COMT

Incubation: According to Axelrod and Tomchick (15), COMT activity was determined by measuring the amount of metanephrine formed from adrenaline. The incubation mixture contained 0.2 ml of crude enzyme preparation, 0.15 μmole of 1-adrenaline d-bitartrate, 5 μmoles of magnesium chloride, 0.25 μmole of S-adenosylmethionine, 25 μmoles of phosphate buffer at pH 7.8, and water to make a final volume of 0.5 ml. The

test compounds in final concentrations of 10^{-3} , 10^{-4} , 10^{-5} M were added to the mixtures. S-Adenosylmethionine was omitted from the reagent blank. The mixtures were incubated in a 40-ml glass-stoppered centrifuge tube at 37°C for 30 minutes. The reaction was stopped by addition of 0.25 ml of borate buffer (0.5 M, pH 10.0).

Extraction of metanephrine: Fifteen ml of isoamyl alcohol, washed previously with 1 N NaOH, 1 N HCl and water, were added to the reaction mixtures, and metanephrine formed was extracted from the aqueous to the organic phase by agitation of the tube for 10 minutes. After centrifugation, 13 ml of the organic phase was transferred to a 20 ml glass-stoppered centrifuge tube containing 1.5 ml of 0.1 N HCl. The tube was agitated for the return of metanephrine from the organic to the aqueous phase. Any residual amount of adrenaline was not extracted to the isoamyl alcohol phase. The average recovery of metanephrine from the HCl phase was about 67% of that formed from adrenaline.

Estimation of metanephrine: The isoamyl alcohol was removed and 0.5 ml of the HCl phase was transferred to a test tube and assayed for metanephrine according to a trihydroxyindole method (16). After addition of 3.3 ml of 0.1 M phosphate buffer (pH 7.2) and 0.05 ml of 1 M NaOH, metanephrine in HCl was oxidized with 0.05 ml of 0.02 M iodine for 5 minutes, then tautomerized by adding 0.5 ml of a mixture of 2% ascorbic acid and 5 N NaOH (1:9). The product was immediately determined by the use of a spectrofluorometer (Hitachi, MPF-2) at the wave length of 517 m μ after activation at 410 m μ . Faded blanks were the samples in which the ascorbic acid was added 5 minutes after the NaOH. The difference between amount of metanephrine formed by incubation with and without S-adenosylmethionine was estimated as enzymatic O-methylation of adrenaline. Each experiment consisted of 3 to 5 observations.

4. Agents tested

dl-2-Amino-phenylethanol sulfate (Aldrich), phenol, *dl*-octopamine hydrochloride (Sigma), *dl*- α -methyloctopamine hydrochloride (Aldrich), *l*-synephrine hydrochloride (Sigma), *l*-phenylephrine hydrochloride (Sigma), pyrocatechol, resorcinol, hydroquinone, pyrogallol, 1, 2, 4-trihydroxybenzene (1, 2, 4-THB, Takeda), phloroglucinol (Roussel), and THPP (Eisai) were tested.

RESULTS

The ability of the crude COMT preparation to methylate adrenaline varied considerably according to the concentration of S-adenosylmethionine added to the incubation media. In the presence of 0.05 μ mole of added methionine the conversion rate of adrenaline to metanephrine was about 29%, while the corresponding value in the presence of 0.25 μ mole of the methionine was 89% (corrected for the recovery of extraction of metanephrine formed).

Using 27.42 μ g (0.15 μ mole) of adrenaline bitartrate as a substrate and 0.25 μ mole of S-adenosylmethionine as a methyl donor, the effects of compounds on the rat liver enzyme activity were determined. Figs. 1-4 show the amounts of metanephrine formed in the presence of test compounds. *dl*-2-Amino-1-phenylethanol and phenol did not inhibit the

FIG. 1. Effect of *dl*-2-amino-1-phenylethanol and phenol on rat liver COMT activity.

FIG. 2. Effect of *dl*-α-methyloctopamine, *dl*-octopamine, *l*-synephrine and *l*-phenylephrine on rat liver COMT activity.

FIG. 3. Effect of pyrocatechol, resorcinol and hydroquinone on rat liver COMT activity.

FIG. 4. Effect of pyrogallol, 1,2,4-THB, phloroglucinol and THPP on rat liver COMT activity.

enzyme activity. Phenolic ethanolamines such as *dl*-octopamine, *dl*- α -methyloctopamine, *l*-synephrine and *l*-phenylephrine slightly inhibited the enzyme activity only at a concentration of 10^{-3} M. Dihydroxybenzenes such as pyrocatechol and hydroquinone definitely inhibited the enzyme activity, but resorcinol was without effect at all concentrations tested. Trihydroxybenzenes, particularly pyrogallol and 1, 2, 4-THB inhibited the COMT activity even at a low concentration of 10^{-6} M. Phloroglucinol and THPP showed weaker but definite inhibitory action on the COMT activity.

DISCUSSION

The present results show that the benzenes which possess two or three adjacent hydroxy groups in the ring inhibit to a various degree the activity of rat liver COMT. Phloroglucinol and THPP also had the COMT inhibitory action *in vitro*. Pharmacological studies of phloroglucinol (5, 11, 12) and THPP (6, 12, 13) showed that these compounds relieve experimental spasms of biliary and urogenital tracts and that they potentiate the responses to exogenously administered catecholamines and to sympathetic nerve stimulation. In the smooth muscles of these organs there is abundant distribution of catecholamine-fluorescent nerve fibers (17). These facts suggest that trihydroxybenzenes exert the spasmolytic effect due to the inhibition of tissue COMT activity with a subsequent increase of sympathetic tone. However, studies were made on the liver enzyme, but not on the COMT of the smooth muscle organs themselves.

At present, there is no available information regarding the role of COMT played in the inactivation of catecholamines in biliary and urogenital organs. Even if it is allowed that the COMT plays an important role in the metabolism of endogenous catecholamines, the exogenously administered hydroxybenzenes must cross the cell membrane before they may attack the enzyme and exert the pharmacological actions. There is another possibility that the hydroxybenzenes exert the pharmacological actions by disturbing the membrane transport mechanism rather than inhibiting the COMT. Before correlating the spasmolytic action of hydroxybenzenes with the inhibition of tissue COMT, it is required to show the increase in level of the endogenous catecholamines and also the decrease in level of metanephrine and normetanephrine in these organs.

SUMMARY

The effects of hydroxybenzenes and related compounds on the activity of rat liver COMT were studied *in vitro*. *dl*-Phenylethanolamine, phenol and resorcinol were without effect. Hydroxyphenylethanolamines and hydroquinone inhibited the enzyme activity only at a high concentration of 10^{-3} M. Pyrogallol and 1, 2, 4-THB showed the strongest inhibition of the enzyme. The effects of pyrocatechol, phloroglucinol and THPP were intermediate. A correlation between spasmolytic actions and COMT inhibition of hydroxybenzenes is discussed.

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