absence of LPL activity found in treated rabbits (65 mg/kg); therefore, we tried to dose this enzyme in adipose tissue.

LPL is thought to be synthesized in various extra-hepatic tissues among which adipose tissue and secreted by cells before being transported to a site at the luminal surface of the capillary endothelial cells where it exerts its activity [20]. LPL activity in perirenal adipose tissue in treated rabbit was similar to that of control rabbits. These experiments permitted us only to estimate a difference between adipose LPL of control and treated rabbits; for real activity values LPL purification could be used. Therefore, it was possible that LPL would be normally synthesized and activated in the adipocyte. A defect of LPL secretion, resulting from treatment, is followed by an accumulation of this enzyme in adipose tissue. LPL would exist in adipocyte under a proenzyme form which would be activated in this cell before being secreted [21–23]. In our experiment, the synthesized LPL would pass from the proenzyme stage to an active enzyme which was never secreted: a change in the adipocyte membrane or in an LPL active structure could be the cause. Further experiments using isolated adipocytes should be carried out to confirm this hypothesis.

In short, the patterns of separation of hepatic triglyceride lipase and lipoprotein lipase by chromatography on heparin-sepharose showed that extra-hepatic lipoprotein lipase was deficient in treated rabbit plasma. The effect of apolipoproteins of molecular weights of about 10,000 on control rabbit post-heparin plasma hepatic triacylglycerol lipase and extra-hepatic lipase was observed. These apolipoproteins inhibited both activities *in vitro*. By their inhibitory effect on these enzymes, these apolipoproteins might play a great part in the development of hypertriglyceridemia after cyclophosphamide injection.

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Biochemical Pharmacology, Vol. 34, No. 19, pp. 3599–3601, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Involvement of catechol-*O*-methyl transferase in the metabolism of the putative dopamine autoreceptor agonist 3-PPP(3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine)

(*Received* 5 *March* 1985; *accepted* 13 *May* 1985)

3-PPP(3-(3-hydroxyphenol)-*N*-*n*-propylpiperidine) has been suggested to act as an agonist at dopamine autoreceptors in the rat brain [1, 2], raising the possibility that this compound may be a potential anti-psychotic agent in man [3]. The compound has a rather low bioavailability in the rat with the bulk of the orally administered 3-PPP being excreted as 3-PPP-glucuronide in the urine [4]. A recent study by Rollema and Mastebroek [5] has also suggested that 3-PPP is hydroxylated to its catechol analogue 4-hydroxy-3-PPP by microsomal enzymes. The authors could demonstrate the presence of 4-hydroxy-3-PPP after incubation of rat liver microsomes with 3-PPP *in vitro*, but *in vivo* the catechol was found in the brain only after treatment of the animals with tropolone, a catechol-*O*-methyl transferase inhibitor [5], raising the possibility that 4-hydroxy-3-PPP is a substrate for this enzyme. This possibility has been investigated in the present study.

Materials and methods

Catechol-*O*-methyl transferase (EC 2.1.1.6, COMT) was prepared from ox liver by the method of Gulliver and Tipton [6] up to, and including, the gel-filtration step. COMT activity was assayed spectrophotometrically at 37° by the coupled assay of Coward and Wu [7] as modified by Gulliver and Tipton [6], in which the *S*-adenosylhomocysteine formed in the reaction is converted to *S*-inosylhomocyteine by the action of adenosine deaminase (EC 2.5.4.4). The reaction mixture contained, in a total volume

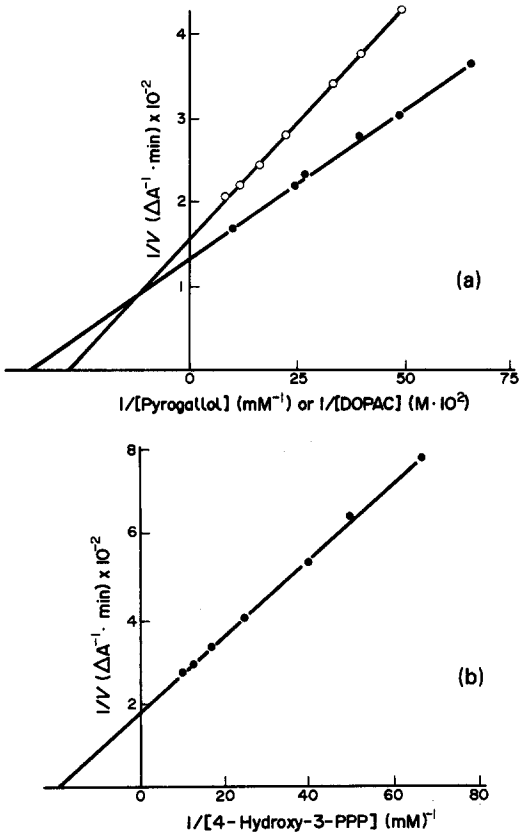

Fig. 1. Double-reciprocal plots of the *O*-methylation by catechol-*O*-methyltransferase of (a) DOPAC (○) and pyrogallol (●) and (b) 4-hydroxy-3-PPP. Each experimental value is the mean of 3 determinations which differed by no more than 5%.

of 500 μl: 200 mM triethanolamine–HCl buffer, pH 7.2, 1.6 mM $MgCl_2$, 0.45 mM *S*-adenosylmethionine, 0.64 units of adenosine deaminase, substrate and the COMT preparation. The decrease in absorbance at 265 nm was followed in a 2 mm path length cuvette. Kinetic parameters were determined by non-linear regression [8, 9]. 4-Hydroxy-3-PPP (LEA 51) was synthesized by Dr. L.-E. Arvidsson, Department of Organic Pharmaceutical Chemistry, BMC, University of Uppsala, Sweden.

Results and discussion

4-Hydroxy-3-PPP was found to be a good substrate for ox liver COMT. The kinetic parameters determined for ox liver COMT with 4-hydroxy-3-PPP are compared with those for 3,4-dihydroxyphenylacetic acid (DOPAC) and pyrogallol in Table 1 and the data from which these were obtained are shown, in double-reciprocal form, in Fig. 1. Although pyrogallol is often used as an inhibitor of COMT,

Table 1. Kinetic constants for substrate methylation by catechol-*O*-methyltransferase

Substrate	K_m (μM)	Maximum velocity (nmoles/min/mg protein)
4-Hydroxy-3-PPP	56 ± 1	32.5 ± 2.4
DOPAC	306 ± 37	35 ± 1.6
Pyrogallol	26 ± 3	44 ± 1.8

Data are given as means (±S.E.) for determinations in three experiments. The values were calculated from the original data (shown in double reciprocal form in Fig. 1) by non-linear regression analysis.

it is in fact a very good substrate for the enzyme and exerts its effects by competing in that way (see [10]). Interestingly, 4-hydroxy-3-PPP has rather similar kinetic parameters to pyrogallol, and both substrates have considerably lower K_m values than DOPAC (Table 1). The latter value is similar to that reported for the enzyme from pig liver [6] and brain [11] and also with the ranges reported by several workers for noradrenaline and dopamine as substrates for this enzyme (see [10]).

In order to establish that the same enzyme was responsible for the methylation of these substrates, the mixed-substrate method (see [12]) was used. The enzyme activity was determined with each substrate separately at its K_m concentration and then with a mixture of two substrates both at their K_m concentrations. If different enzymes were responsible for the reactions, the velocity determined with the mixture would be expected to be equal to the sum of the velocities determined with the two substrates assayed separately, whereas, if the same enzyme were responsible for the activity towards both substrates, the activity with the mixture would be expected to be only 66.7% of the sum of the activities with the two substrates separately. The data shown in Table 2 show that the velocities observed for the substrate pairs are consistent with a single enzyme being responsible for their methylation.

In conclusion, the present study has demonstrated 4-hydroxy-3-PPP to be a good substrate for COMT with a K_m value similar to that found for pyrogallol and considerably lower than that found for DOPAC. The immunological similarity between brain and liver COMT [11] might suggest that methylation would also occur in the CNS. However, this might depend on accessibility to the intracellular COMT. It has previously been reported that 4-hydroxy-3-PPP has dopamine agonist properties both at pre- and post-synaptic sites, but that the presynaptic action is three times less potent than that for 3-PPP [3].

It is possible that 4-hydroxy-3-PPP is in fact more potent than suggested by this test, but that the compound is metabolized so extensively by COMT that its availability at the receptor site(s) is rather poor. Such a hypothesis could easily be tested by giving the drug to animals pre-treated with a COMT inhibitor. As well as having dopamine agonist actions, the presence of sufficient amounts of 4-hydroxy-3-PPP in the brain will, in a manner analogous

Table 2. Activities of catechol-*O*-methyltransferase towards substrate mixtures

Substrate	Sum of individual velocities	Observed velocity (%)
DOPAC + 4-Hydroxy-3-PPP	35.2	23.7 ± 1.0 (67)
Pyrogallol + 4-Hydroxy-3-PPP	35.5	22.7 ± 0.6 (62)

Activities were determined at K_m concentrations of each substrate separately and then with the mixtures of each at the same concentrations. Initial velocities are expressed as nmol/min/mg protein and the observed velocities are mean values ± S.E.M. of 3 separate determinations.

to that found with pyrogallol (see [10]), prevent the *O*-methylation of not only DOPAC but also biogenic amines by COMT. However, it is unlikely that such high concentrations will be reached after 3-PPP treatment.

Rollema and Mastebroek [5] have suggested that the final product after the metabolism of 3-PPP first by the liver hydroxylating systems and then by COMT is 4-methoxy-3-PPP. However, since COMT generally methylates at the hydroxyl group *meta* to the substituent (although *para*-methylation has been found under certain conditions, see [13]), it is perhaps more likely that the final product is 3-(3-methoxy-4-hydroxyphenyl)-*N*-*n*-propylpiperidine. Needless to say, the position of the methylation can only be ascertained with certainty by isolating the product and comparing its chemical properties with chemically synthesized meta- and para-*O*-methylated 4-hydroxy-3-PPP.

Acknowledgements—We are grateful to Dr. L.-E. Arvidsson for his gift of a sample of LEA 51, to Dr. J. Lundström for permission to cite his unpublished work and to the M.R.C.I. for support.

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Biochemical Pharmacology, Vol. 34, No. 19, pp. 3601–3603, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Protection against alloxan-induced diabetes in mice by the free radical scavenger butylated hydroxyanisole

(*Received* 28 *January* 1985; *accepted* 13 *May* 1985)

Recent research has clearly demonstrated the role played by free radicals in various important pathological processes [1, 2] and high-lighted the need for safe and effective inhibitors of free radical-induced tissue injury. Alloxan-induced damage to pancreatic β-cells, an example of this type of pathology [3, 4], can be inhibited *in vivo* by aliphatic hydroxyl radical (·OH) scavengers such as dimethylsulfoxide (DMSO)* [5], *N*,*N*′-dimethylurea (DMU) [6], thiourea [7], and the short chain aliphatic alcohols, e.g. *n*-butanol [7]. While such scavengers strongly indicate a role for ·OH in alloxan-induced diabetes, their general usefulness for preventing free radical-induced tissue injury is limited. They must, for example, be administered in large doses (g/kg) to give protection in this system [5–7] and others [8, 9]. Furthermore, in the process of scavenging ·OH some of these aliphatic compounds may form potentially damaging free radical by-products [10] or other toxic metabolites [11].

Butylated hydroxyanisole (BHA), a phenolic resonance-stabilised free radical scavenger [12], is sufficiently non-toxic to be used as a food additive [13]. Since phenols have a higher rate constant for reaction with ·OH than the above aliphatic substances [14, 15] and the free radical formed by BHA when it scavenges is not a reactive or damaging species [12], we predicted that BHA should be an effective low dose inhibitor of alloxan-induced diabetes.

* Abbreviations used: BHA, butylated hydroxyanisole; DMSO, dimethylsulfoxide; DMU, *N*,*N*′-dimethylurea.

Materials and methods

Male CBA mice between 8 and 10 weeks old and weighing 23–27 g were used. Alloxan monohydrate (Sigma Chemical Co., St. Louis, MO) was dissolved in normal saline at 6.25 mg/ml. BHA (Sigma Chemical Co.) was dissolved in pure olive oil (Faulding) at 15, 20, 25 and 30 mg/ml.

Mice were starved for 6 hr prior to receiving intraperitoneal (i.p.) injections of 0.1 ml saline, 0.1 ml olive oil or 0.1 ml olive oil containing 1.5, 2.0, 2.5 or 3.0 mg of BHA. One, two or three hours later intravenous (i.v.) injections of 0.2 ml saline or 0.2 ml saline containing 1.25 mg of alloxan monohydrate were given.

Except where otherwise stated, plasma from each mouse was assayed for glucose at the time of the first injection and 1, 24, 48, 72 and 96 hr afterwards. For each assay about 50 μl of blood was taken from the tail vein of each mouse into heparinised microtubes (Clay Adams). These were sealed and spun for 3 min in a microtube centrifuge (Clements) to collect plasma. Plasma glucose (mmol/l) was assayed using the GOD-Perid Test Combination (Boehrin-