J. steroid Biochem. Vol. 17, pp. 17 to 21, 1982
Printed in Great Britain. All rights reserved

0022-4731/82/010017-05$03.00/0
Copyright © 1982 Pergamon Press Ltd

2-HYDROXYETHYNYLOESTRADIOL AS A SUBSTRATE FOR CATECHOL-*O*-METHYLTRANSFERASE—IMPLICATIONS IN THE METABOLISM OF ETHYNYLOESTRADIOL

MICHAEL J. RAXWORTHY and PETER A. GULLIVER*
Department of Pharmacology, Worsley Medical and Dental Building, The University, Leeds, LS2 9JT, U.K.

(*Received* 29 *July* 1981)

SUMMARY

Highly purified pig catechol-*O*-methyltransferase catalyses the methylation of 2-hydroxyethynyloestradiol ($K_M = 11.0\ \mu M$, $V_{max} = 521.2$ mU/mg protein, $V_{max}/K_M = 47.4$) more efficiently than that of 2-hydroxyoestradiol ($K_M = 68.5\ \mu M$, $V_{max} = 1056.2$ mU/mg protein, $V_{max}/K_m = 15.4$), 2-hydroxyoestrone ($K_M = 38.0\ \mu M$, $V_{max} = 795.0$ mU/mg protein, $V_{max}/K_M = 20.9$) or 4-hydroxyoestrone ($K_M = 12.8\ \mu M$, $V_{max} = 159.7$, $V_{max}/K_M = 12.5$). This efficient methylation of the principal metabolite of ethynyloestradiol substantiates the implications of the studies of Bolt *et al.*[1] that *O*-methylation is a major route of ethynyloestradiol metabolism. Furthermore, this also implies that catechol-*O*-methyltransferase is involved in the protection, by S-adenosylmethionine, against the impairment of bile secretion by ethynyloestradiol, observed in female rats [2].

INTRODUCTION

The aromatic ring of oestrogens can be hydroxylated at the 2 and 4 positions by cytochrome P450-dependent hydroxylases. The former is a major route of metabolism of these hormones [3, 4] and may represent a potential control mechanism since catecholoestrogens have been shown to have different effects from the parent compounds [5–7] whilst still retaining an affinity for the oestrogen receptors of the hypothalamus and pituitary comparable to the parent compounds [8]. The formation, localisation and physiological role of catecholoestrogens have recently been reviewed [9, 10]. Similarly, ethynyloestradiol, the contraceptive hormone, is also metabolised by hydroxylation mainly at the 2- position, giving rise to a catechol structure [1].

Catechol-*O*-methyltransferase (COMT, E.C.2.1.1.6) catalyses the methylation of a very wide range of catechol substrates using S-adenosylmethionine as the methyl donor in the presence of magnesium ions (see [11] for review). When first discovered it was assumed that the natural COMT substrates were the catecholamines and their metabolites [12], however, further work showed that COMT catalysed the methylation of catecholoestrogens *in vitro* [13] and *in vivo* [14].

Previous workers, however, used impure COMT preparations and assays were carried out in the absence of adenosine deaminase, which leads to a build-up of the inhibitory co-enzyme product S-adenosylhomocysteine. This has been shown to cause deviations from initial rate kinetics [15]. In this study we use a highly purified homogeneous COMT preparation in the adenosine deaminase-coupled spectrophotometric assay which enables initial rates to be determined more accurately, at optimal pH and magnesium cofactor concentration.

EXPERIMENTAL

All reagents were of analytical quality (BDH, Poole, U.K.) and were dissolved in glass distilled water. All pH measurements are relative to 20°C.

Catechol-*O*-methyltransferase was purified from deep frozen pig liver by homogenization, ammonium sulphate precipitation from the 22,190 *g* supernatant, chromatography of the redissolved precipitate on Sephadex G75 and affinity chromatography on 2,6-dimethoxyphenol-azo-phenyl-methylene-anilino-agarose as in [16] but with a modified buffer [17]. During purification COMT activity was assayed radiochemically [15]: one unit (U) of activity represents the formation of one micromole of product per min at 37°C. Protein concentrations above 1 mg/ml were determined by the biuret method [18] and, at lower concentrations, by the tannate method [19] using standard curves prepared to bovine serum albumin (Sigma, Poole, U.K.).

The COMT preparation used had a specific activity of 1375 mU/mg protein representing an overall purification of 914-fold compared to the homogenate. Purification details of this preparation are given in [20]. Purity of COMT prepared by this method from pig liver has previously been investigated by polyacrylamide gel electrophoresis [16]: only one protein was detected. It should be noted that the specific activity

* To whom correspondence should be sent.

Table 1. Apparent kinetic constants of pig liver COMT for catechol oestrogens*

Substrate	Concentration range (μM)	Apparent K_M (μM)	Apparent V_{max} (mU/mg protein)	Apparent first order rate constant (V_{max}/K_M)
2-Hydroxyoestradiol†	9.8–106.4	68.5	1056.2	15.4
2-Hydroxyoestrone†	15–58	38.0	795.0	20.9
4-Hydroxyoestrone	12.8–147.3	12.8	159.7	12.5
2-Hydroxyethynyloestradiol	6.1–46.1	11.0	521.2	47.4

* Determined by the method of Coward and Wu[21] as modified in Gulliver and Tipton[15] in the presence of 1.6 mM magnesium chloride, 0.456 mM S-adenosylmethionine and 0.20 M triethanolamine hydrochloride buffer pH 7.20, at 37°C.
† Consistent deviation from Michaelis–Menten kinetics of the substrate inhibition type observed with this substrate at high concentrations.

of the COMT preparation used in this study is higher than obtained previously [16, 17] implying homogeneity.

The investigation of catecholoestrogens as COMT substrates utilised the coupled spectrophotometric assay of Coward and Wu[21] as modified in [15]. The assay mixture consisted of 1.6 mM magnesium chloride, 0.64 U adenosine deaminase (purified from Takadiastase up to and including the dialysis step of Sharpless and Wolfenden[22]), 0.20 M triethanolamine hydrochloride buffer pH 7.20, 0.456 mM S-adenosylmethionine, a catecholoestrogen in 3–35 μl ethanol and 5–25 μl (4.3–21.5 mU) purified COMT, in a final volume of 500 μl. The pH and magnesium concentrations are both optimal [15, 16]. Initial rates of decrease in absorbance at 265 nm were measured at 37°C in a Beckman Model 35 spectrophotometer. The quantity of ethanol used as solvent for the catecholoestrogens did not affect the rates obtained.

Determinations were replicated at least eight times at each concentration of catecholoestrogen, the median used in kinetic analysis, and the widest possible range of substrate concentrations employed. The apparent kinetic constants, K_M and V_{max}, were determined by the direct linear plot [23] and shown to have a confidence value within the 95% region by a non-parametric method [24, 25].

2-hydroxyoestradiol (oestra-1,3,5(10)triene-2,3,17β-triol), 2-hydroxyoestrone (2,3-dihydroxyoestra-1,3,5(10)-trien-17-one) and 4-hydroxyoestrone (3,4-dihydroxyoestra-1,3,5(10)-trien-17-one) were obtained from Professor D. N. Kirk, MRC Steroid Reference Collection, Westfield College, London, U.K. Purity of these compounds has been demonstrated at the MRC Steroid Reference Collection by gas-liquid chromatography of the trimethyl silane derivatives performed on a Packard 429 instrument using a capillary column: less than 2% contamination was detected.

2-Hydroxyethynyloestradiol (17α-ethynyloestra-1,3,5(10)-triene-2,3,17-triol) was a gift from Dr P. Narashimha Rao, Southwest Foundation, San Antonio, Texas U.S.A. Oxidation of the substrates during the assay can be detected by a rising spectrophotometric baseline. No such deterioration was observed due to the protection afforded by the thiol reducing agents present in the enzyme preparation. Gelbke and Knuppen[26] have shown that ascorbic acid is most effective in preventing oxidative decomposition of catecholoestrogens but its use in the spectrophotometric assay employed here is precluded due to its absorbance peak at 265 nm and its reported ability to act as a COMT substrate [27].

The lack of oxidation and the use of initial rate determinations to ascertain kinetic parameters suggest that results are not influenced by degradation of the catecholoestrogens.

RESULTS

The apparent kinetic constants obtained in this study are given in Table 1. 2-Hydroxyoestradiol and 2-hydroxyoestrone showed, at high concentrations, consistent deviations from Michaelis–Menten kinetics of the substrate inhibition type as observed previously with some catecholamine derivatives ([17] and see Table 2 derived from [20]).

The apparent first order rate constants (V_{max}/K_M) have been computed. This is proportional to the rate observed at low, and probably physiological, concentrations of the substrates. By this criterion, 2-hydroxyethynyloestradiol is by far the most avidly utilised catecholoestrogen substrate.

DISCUSSION

Catechol-*O*-methyltransferase has a great avidity for catecholoestrogens as substrates. The K_M values obtained are an order of magnitude lower than those for catecholamines and derivatives ([17] and see Table 2 derived from [20]); indeed, the constants suggest that COMT has a greater affinity for some of these compounds than the coenzyme substrate, S-adenosylmethionine (K_M = 56 μM, [16]; 35 μM [17]).

Extensive kinetic data for the three natural catecholoestrogens investigated in the present study have been presented for COMT from rat [28] and human liver [5]. Human liver appears to have a K_M value twice that of rat liver for these substrates but only small differences in K_M within species were observed with these catecholoestrogens. The K_M estimated for 4-hydroxyoestrone with pig liver COMT is inter-

Table 2. Apparent kinetic constants of pig liver COMT for catecholamines and metabolites*

Substrate	Concentration range (μM)	Apparent K_M (μM)	Apparent V_{max} (mU/mg protein)	Apparent first order rate constant (V_{max}/K_M)
(−)Adrenaline†	180–960	510	590.9	1.16
(−)Noradrenaline†	120–1200	520	342.8	0.66
Dopamine	360–1800	750	157.5	0.21
L-DOPA⁺	720–4300	1700	291.9	0.17
3,4-dihydroxyphenylacetic acid	360–1440	680	778.3	1.14

Data obtained with the same preparation of COMT taken from Gordonsmith *et al.*[20].

* Determined by the method of Coward and Wu[21] as modified in Gulliver and Tipton[15] in the presence of 1.6 mM magnesium chloride, 0.456 mM S-adenosylmethionine and 0.20 M triethanolamine hydrochloride buffer pH 7.20, at 37°C.

† Consistent deviation from Michaelis–Menten kinetics of the substrate inhibition type observed with this substrate at high concentrations.

mediate between the values of [28] and [5], however, a different relationship between the affinities of 2-hydroxyoestradiol and 2-hydroxyoestrone as compared with the affinity of 4-hydroxyoestrone is indicated in the present study. These affinities were approximately 5- and 3-fold lower than 4-hydroxyoestrone for 2-hydroxyoestradiol and 2-hydroxyoestrone respectively. Differences in the degree of purification of the enzyme preparations used do not permit valid comparisons of V_{max} values.

A number of factors may contribute to the disparity in kinetic data for 2-hydroxyoestradiol and 2-hydroxyoestrone. Merriam *et al.*[28] using a non-linear curve fitting program to compute Michaelis constants did not observe substrate inhibition. However this type of inhibition has been reported for the 2-hydroxyoestrogens [5] and its characteristic pattern was observed by the direct linear plot of Eisenthal and Cornish-Bowden[23] which has proved capable of showing substrate inhibition by catecholamines and derivatives [17, 20]. If substrate inhibition does occur with 2-hydroxyoestrogens but was not detected by Merriam *et al.*[28] then inclusion of data at high substrate concentrations will tend to lower the K_M estimate.

The absence of adenosine deaminase from the assays used in [5] and [28] will also tend to cause the over-estimation of affinity of catecholoestrogens for COMT due to deviation from linearity, and hence proportionality, as the concentration of the inhibitory product S-adenosylhomocysteine increases during the assay. The addition of ascorbic acid to the assay of Merriam *et al.*[28] is a further complication since it has been reported to be a COMT substrate [27].

These factors plus the respective purities of the enzyme preparations (914-fold pig liver COMT—this study; 380-fold human liver COMT [5]; rat liver COMT [28]—purified by the method of Coyle and Henry[29]; our method [16] has additional ammonium sulphate precipitation, Sephadex G75 and affinity chromatography steps) and possible species differences mean that comparison of kinetic data must be approached with caution and echo the difficulties encountered by Guldberg and Marsden[11] in this respect.

The avidity of COMT for catecholoestrogens coupled with the wide distribution of COMT in target tissues such as uterus [30, 31], breast [32] and hypothalamus [33, 34] imply that the access of oestrogen to their receptors in these tissues could be controlled by hydroxylation and subsequent methylation. Paul and Axelrod[7] have shown that the levels of catecholoestrogens in the hypothalamus and pituitary are ten-fold greater than those of the parent compounds. However, this finding has been disputed [9].

2-Hydroxyoestrone is not uterotrophic but differs from other oestrogens in suppressing prolactin release whereas 17-β-oestradiol stimulates release [35] thus demonstrating that catecholoestrogens have their own distinctive functions. COMT may therefore have a role in modulating prolactin and gonadotrophin action by controlling plasma 2-hydroxyoestrone levels.

Difficulties in interpreting kinetic data for natural catecholoestrogens notwithstanding, perhaps the result of major interest from the present study is the finding that 2-hydroxyethynyloestradiol is preferred as a substrate of COMT to these natural metabolites.

We have confirmed the implication [1] that 2-hydroxylation of ethynyloestradiol can be followed by *O*-methylation catalysed by COMT. Our findings also explain the effect observed by Stramentinoli *et al.*[2] that the impairment of bile secretion by ethynyloestradiol [36] is overcome by treatment with S-adenosylmethionine since this stimulates methylation of the 2-hydroxy metabolite of ethynyloestradiol. Ethynyloestradiol administration has been shown to result in the irrevesible binding of a chemically reactive metabolite to rat liver microsomal protein *in vitro* [37, 38]. This metabolite has been postulated to be a quinone or semi-quinone derived from 2-hydroxyethynyloestradiol. Irreversible binding results in the reduction of bile flow by reducing both bile salt dependent and independent fractions of the bile [39]. It is thought that *O*-methylation of 2-hydroxyethynyloestradiol prevents its oxidation and in turn prevents microsomal proteins binding [37] hence methylated metabolites are eliminated faster. Our results show conclusively that the methylation of 2-hydroxyethynyloestradiol is catalysed very rapidly by COMT.

COMT may thus play an important part in the protection against the inhibitory effects of ethynyloestradiol on bile secretion.

Finally, there are profound implications for our understanding of catecholamine metabolism: high catecholoestrogen levels would inhibit *O*-methylation of catecholamines by COMT by acting as excellent competitive inhibitors and, furthermore, cause a decrease in the limited S-adenosylmethionine pool [40]. This may have implications in pregnancy (see [5]) and in the use of oral contraceptives. Experimentally, 17β-oestradiol is widely used in various isolated tissue preparations to inhibit the catecholamine Uptake 2 system (see [41] for review). This compound may exert its effect by undergoing hydroxylation and hence act as a competitive COMT substrate [14]. This may result in the alteration of the role of COMT in the "extraneuronal *O*-methylating system" [41] in which uptake of catecholamines works in series with *O*-methylation. Recently, Köster and Breuer[42], noting the ability of 17β-oestradiol to inhibit methylation of noradrenaline, speculated that the best explanation of their results was that 17β-oestradiol or its metabolites competitively inhibit COMT and that 17β-oestradiol may also play a role in modifying the uptake of noradrenaline.

Acknowledgements—We thank the MRC for financial support, Mr John Savage of Leeds abbatoir for his co-operation and Dr G. Stramentinoli of Bio Research, Milan, Italy for the gift of S-adenosylmethionine.

We thank, especially, Dr R. E. Oakey of the Steroid Endocrinology Laboratory, Department of Chemical Pathology, University of Leeds for his help and encouragement. We also thank Professor D. N. Kirk, MRC Steroid Reference Collection, Westfield College, London, U.K. for his advice and help and along with Dr P. Narasimha Rao, Southwest Foundation, San Antonio, Texas, U.S.A., for generous gifts of catecholoestrogens.

REFERENCES

1. Bolt H. N., Kappus H. and Remmer H.: Studies on the metabolism of ethynylestradiol *in vitro* and *in vivo*; the significance of 2-hydroxylation and the formation of polar products. *Xenobiotica* **3** (1973) 773–785.
2. Stramentinoli G., DiPadova C., Gualano M., Rovagnati P. and Galli-Kienle M.: Ethynylestradiol-induced impairment of bile secretion in the rat: protective effects of S-adenosyl-L-methionine and its implication in estrogen metabolism. *Gastroenterology* **80** (1981) 154–158.
3. Fishman J.: Role of 2-hydroxyestrone in estrogen metabolism. *J. clin. Endocr. Metab.* **23** (1963) 207–210.
4. Ball P., Hoppen H-O. and Knuppen R.: Metabolism of oestradiol-17β and 2-hydroxy-oestradiol-17β in rat liver slices. *Hoppe-Seyler's Z. Physiol. Chem.* **355** (1974) 1451–1462.
5. Ball P., Knuppen R., Haupt M. and Breuer H.: Interactions between estrogens and catecholamines III: Studies on the methylation of catechol estrogens, catecholamines and other catechols by the catechol-*O*-methyltransferase of human liver. *J. clin. Endocr. Metab.* **34** (1972) 736–746.
6. Paul S. M. and Skolnick P.: Catechol-oestrogens inhibit oestrogen elicited accumulation of hypothalamic cAMP suggesting role as endogenous anti-oestrogens. *Nature* **266** (1977) 559–561.
7. Paul S. M. and Axelrod J.: Catecholoestrogens: presence in brain and endocrine tissues. *Science* **97** (1977) 657–659.
8. Davies I. J., Naftolin F., Ryan K. J., Fishman J. and Sie J.: The affinity of catecholestrogens for estrogen receptors in the pituitary and anterior hypothalamus of the rat. *Endocrinology* **97** (1975) 554–557.
9. Ball P. and Knuppen R.: Catecholoestrogens (2- and 4-hydroxyoestrogens): chemistry, biogenesis, metabolism, occurrence and physiological significance. *Acta endocr., Copenh.* **93** (1980) Supplement 232 1–128.
10. Chao S. T., Omiecinski C. J., Nankung M. J., Nelson S. D., Dvorchik B. H. and Juchau M. R.: Catechol estrogen formation in placental and fetal tissues of humans, macaques, rats and rabbits. *Dev. Pharmacol. Ther.* **2** (1981) 1–16.
11. Guldberg H. C. and Marsden C. A.: Catechol-*O*-methyltransferase—pharmacological aspects and physiological role. *Pharmacol. Revs.* **27** (1975) 135–206.
12. Axelrod J. and Tomchick R.: Enzymic *O*-methylation of epinephrine and other catechols. *J. biol. Chem.* **233** (1958) 702–705.
13. Breuer H., Vogel W. and Knuppen R.: Enzymatische methyliening von 2-hydroxyöstradiol-17β durch eine S-adenosylmethionine acceptor-*O*-methyltransferase der ratten leber. *Hoppe-Seyler's Z. Physiol. Chem.* **327** (1962) 217–224.
14. Knuppen R., Holler M., Tilmann D. and Breuer H.: Interactions between oestrogens and catecholamines II: effect of oestrogens on the degradation of adrenaline in mouse under *in vivo* conditions. *Hoppe-Seyler's Z. Physiol. Chem.* **350** (1969) 1301–1309.
15. Gulliver P. A. and Tipton K. F.: Direct extraction radioassay for catechol-*O*-methyltransferase activity. *Biochem. Pharmacol.* **27** (1978) 773–775.
16. Gulliver P. A. and Tipton K. F.: Purification and properties of pig-liver catechol-*O*-methyltransferase. *Eur. J. Biochem.* **88** (1978) 439–444.
17. Hagan R. M., Raxworthy M. J. and Gulliver P. A.: Benserazide and carbidopa as substrates of catechol-*O*-methyltransferase: new mechanism of action in Parkinson's disease. *Biochem. Pharmacol.* **29** (1980) 3123–3126.
18. Gornall A. G., Bardawill C. J. and David M. M.: Determination of serum proteins by the biuret method. *J. biol. Chem.* **177** (1949) 751–766.
19. Mejbaum-Katzenellenbogen W. and Dobryszycka W. M.: New method for quantitative determination of serum proteins separated by paper electrophoresis. *Clin. chim. Acta* **4** (1959) 515–522.
20. Gordonsmith R. H., Raxworthy M. J. and Gulliver P. A.: Substrate stereospecificity and selectivity of catechol-*O*-methyltransferase for DOPA, DOPA derivatives and α-substituted catecholamines. *Biochem. Pharmacol.* **31** (1981) 433–437.
21. Coward J. K. and Wu F. H-H.: A continuous spectrophotometric assay for catechol-*O*-methyltransferase. *Analyt. Biochem.* **55** (1973) 406–410.
22. Sharpless T. K. and Wolfenden R.: A purified non-specific adenosine deaminase from Taka-diastase. *Met. Enzymol.* (Edited by S. P. Colowick and N. O. Kaplan) **12A** (1967) 126–131.
23. Eisenthal R. and Cornish-Bowden A.: The direct linear plot—a new graphical method of estimating enzyme kinetic parameters. *Biochem. J.* **139** (1974) 715–720.
24. Cornish-Bowden A. and Eisenthal R.: Statistical considerations in the estimation of enzyme kinetic parameters by the direct linear plot and other methods. *Biochem. J.* **139** (1974) 721–730.
25. Cornish-Bowden A. and Eisenthal R.: Estimation of Michaelis constants and maximum velocity from the direct linear plot. *Biochem. biophys. Acta.* **523** (1978) 268–272.

26. Gelbke H. P. and Knuppen R.: A new method of preventing oxidative decomposition of catechol estrogens during chromatography. *J. Chromatogr.* **71** (1972) 465–471.
27. Blaschke E. and Hertting G.: Enzymic methylation of L-ascorbic acid by catechol-*O*-methyltransferase. *Biochem. Pharmacol.* **20** (1971) 1363–1370.
28. Merriam G. R., Maclusky N. J., Picard M. K. and Naftolin F.: Comparative properties of the catecholestrogens: methylation by catechol-*O*-methyl-transferase and binding to cytosol estrogen receptors. *Steroids* **36** (1980) 1–11.
29. Coyle J. T. and Henry D.: Catecholamines in fetal and newborn rat brain. *J. Neurochem.* **21** (1973) 61–67.
30. Wurtman R. J., Axelrod J. and Potter L. T.: The disposition of catecholamines in the rat uterus and the effects of drugs and hormones. *J. Pharmacol. Exp. Ther.* **144** (1964) 150–155.
31. Inoue K., Tice L. W. and Creveling C. R.: Immunocytochemical localization of catechol-*O*-methyltransferase in the pregnant rat uterus. *Endocrinology* **107** (1980) 1833–1840.
32. Assicot M., Contesso G. and Bohuon C.: Catecho-*O*-methyltransferase in human breast cancers. *Eur. J. Cancer* **13** (1977) 961–966.
33. Robinson D. S., Sourkes T. L., Nies A., Harris L. S., Spector S., Bartlett D. L. and Kaye I. W.: Monoamine metabolism in human brain. *Arch. Gen. Psychiat.* **34** (1977) 89–92.
34. Saavedra J. M., Brownstein M. J. and Palkovits M.: Distribution of catechol-*O*-methyltransferase, histamine-*N*-methyltransferase and monoamine oxidase in specific areas of rat brain. *Brain Res.* **118** (1976) 152–156.
35. Fishman J. and Tulchinsky D.: Suppression of prolactin secretion in normal young women by 2-hydroxyesterone. *Science* **210** (1980) 73–74.
36. Heikal T. A. J. and Lathe G. H.: The effect of oral contraceptive steroids on bile secretion and bilirubin T_m in rats. *Br. J. Pharmacol.* **38** (1970) 593–601.
37. Kappus H. and Bolt H. M.: Irreversible binding of ethynyl-oestradiol metabolites to protein: lack of methylation by S-adenosylmethionine. *Horm. Metab. Res.* **6** (1974) 333–334.
38. Breckenridge A. M., Grabowski P. S., Maggs J. L. and Park B. K.: Inhibition of the irreversible binding of ethynyloestradiol in rat liver microsomes *in vitro*. *Br. J. Pharmacol.* **74** (1981) 240P–241P.
39. Stramentinoli G., Gualano M., Rovagnati P. and DiPadova C.: Influence of S-adenosylmethionine on irreversible binding of ethynyloestradiol to rat liver microsomes and its impications in bile secretion. *Biochem. Pharmacol.* **28** (1979) 981–984.
40. Mudd S. H. and Poole J. R.: Labile methyl balances for normal humans on various dietry regimens. *Metabolism* **24** (1975) 721–735.
41. Trendelenburg U.: A kinetic analysis of the extra-neuronal uptake and metabolism of catecholamines. *Rev. Physiol. Biochem. Pharmacol.* **87** (1980) 33–115.
42. Köster G. and Breuer H.: Effect of oestradiol-17β on the metabolism of noradrenaline in brain slices of ovariectomized rats. *Acta endocr., Copenh.* **98** (1981) 1–7.