Biochemical Pharmacology, 1961, Vol. 6, pp. 47–54. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Inhibition of catechol-O-methyl transferase by pyrogallol in the rat

(*Received* 31 *October* 1960)

THE MAJOR route of metabolism of circulating catecholamines in the rat has been shown to involve meta-O-methylation by catechol-O-methyl transferase.[1] S-Adenosylmethionine is the methyl group donor, and the reaction requires a divalent cation.[2] Pyrogallol has recently been shown to be an inhibitor of this enzyme:[3, 4, 5] however the kinetics of this inhibitory action *in vitro* and the effectiveness of pyrogallol *in vivo* as an enzyme inhibitor in the tissues have not been studied in detail. This communication presents data which bear on these topics.

Optimal conditions for the assay of the enzyme were as follows: 2 μmoles of l-norepinephrine, 0·5 μmole of S-adenosylmethionine, 2 μmoles of magnesium chloride, 50 μmoles of sodium phosphate buffer (pH 7·9), and an appropriate amount of enzyme in a total volume of 1·0 ml. After incubation at 37 °C the reaction was stopped by the addition of 0·5 ml of 0·5 M sodium borate buffer (pH 10), and the mixture was assayed fluorimetrically for 3-methoxy norepinephrine.[2] The reaction rate was not altered by greater than optimal concentrations of substrate or S-adenosylmethionine, but magnesium concentrations above 5×10^{-3} M were inhibitory. Under optimal conditions the rate was linear with time for only 20 min. In previous studies of the kinetics of this enzyme, lower concentrations of S-adenosylmethionine and 30–90 min periods of incubation were used.[2, 6] In our system valid kinetic data could not be obtained under these conditions.

Catechol-O-methyl transferase was purified approximately 30-fold from the liver, brain, and heart of the rat, as described by Axelrod and Tomchick.[2] The rate of norepinephrine metabolism by these preparations is shown in Fig. 1. The K_m for norepinephrine was approximately 3×10^{-4} M, regardless of the source of the enzyme. This differs from previous reports that the K_m for epinephrine is $1{\cdot}2 \times 10^{-4}$ M for the liver enzyme,[2] but less than 10^{-5} M in brain.[6] The K_m for S-adenosylmethionine was found to be 4×10^{-5} M.

Pyrogallol is known to be a substrate for catechol-O-methyl transferase,[7] and its inhibitory action has been described as competitive.[3] As illustrated in Fig. 1, however, the inhibition of norepinephrine metabolism by pyrogallol appears in this system to be at least partially non-competitive. The degree of non-competitive inhibition was greatest in the most purified enzyme preparations and increased slightly if the enzyme was pre-incubated with pyrogallol and substrate before the addition of S-adenosylmethionine. In the experiment described in Fig. 1, a partially purified (30-fold) liver preparation and a 5-min pre-incubation period were employed. The K_i for pyrogallol was 8×10^{-6} M under these conditions.

The degree of enzyme inhibition produced by pyrogallol *in vivo* was estimated in various tissues of the rat at several time intervals following the intraperitoneal injection of the inhibitor. Tissues were homogenized in 1 vol. of cold 0·1 M sodium phosphate buffer (pH 7·9) containing 2·5 μmoles of magnesium chloride per ml. The homogenate was centrifuged at 15,000 **g** for 20 min, and the supernatant fluid containing the soluble

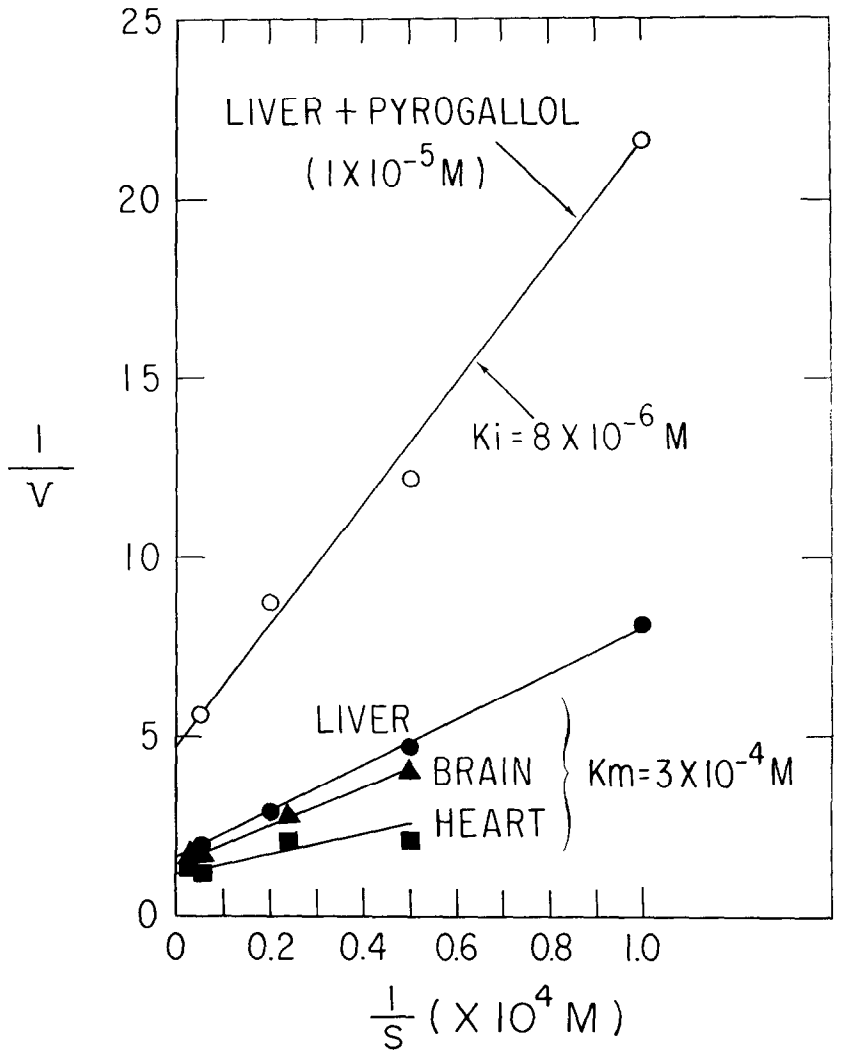

FIG. 1. Rate of norepinephrine metabolism *in vitro* by catechol-O-methyl transferase from three separate organs of rat; inhibition of liver enzyme by pyrogallol (data plotted by method of Lineweaver and Burk). Liver enzyme (0·48 mg protein) incubated 10 min; brain (1·8 mg protein) and heart (2·0 mg protein) preparations incubated 15 min; v is expressed as μ moles/hr per 1 mg protein for liver, per 15 mg protein for brain, and per 75 mg protein for heart, in order to fit all curves conveniently on one graph.

enzyme was removed for assay. Little or no activity was found in the particulate sediment. The incubation beaker contained 0·9 ml of the supernatant fluid, 2 μmoles of l-norepinephrine, and 0·5 μmole of S-adenosylmethionine in a volume of 1·0 ml. Dilution of the inhibitor from its concentration in tissue was thus maintained at a minimum. The percentage inhibition was determined by comparing the activity of tissues from pyrogallol-treated rats with that from control animals. In these concentrated supernatant solutions some activity (10–40 per cent of maximal) could be detected in control brain and heart without the addition of S-adenosylmethionine. The degree of inhibition was determined in the presence of both optimal concentrations and endogenous concentrations of co-factor (Table 1). The inhibitory effects of

TABLE 1. *In vivo*-INHIBITION OF CATECHOL-O-METHYL TRANSFERASE IN THE LIVER, BRAIN, AND HEART OF THE RAT FOLLOWING THE INTRAPERITONEAL INJECTION OF PYROGALLOL

Dose	Organ	Percentage Inhibition*					
		No added *S-adenosylmethionine*			With added *S-adenosylmethionine*		
		15 min	30 min	60 min	15 min	30 min	60 min
Single injection:							
50 mg/kg	Liver	—	—	—	78	41	17
	Brain	100	65	—	44	31	9
	Heart	100	70	—	48	32	3
200 mg/kg	Liver	—	—	—	—	96	84
	Brain	—	100	100	—	78	63
	Heart	—	100	100	—	81	73
Repeated injection:							
50 mg/kg per 30 min for 3 hr†	Liver	—	—	—	90	—	—
	Brain	100	—	—	62	—	—
50 mg/kg per 30 min for 16 hr†	Liver	100	—	—	90	—	—
	Brain	100	—	—	76	—	—
	Heart	100	—	—	67	—	—

* See text for details of procedure.

† Rats injected every 30 min and sacrificed 15 min after the last dose.

pyrogallol were of short duration *in vivo*, but effective inhibition of the enzyme could be achieved for 30–60 min with a single dose of 200 mg/kg. A repeated dose of 50 mg/kg every 30 min was required to maintain more prolonged enzyme inhibition.

These studies confirm that pyrogallol is a potent inhibitor of catechol-O-methyl transferase. Although this agent probably acts in large part as a competitive substrate, a non-competitive component of its action can be demonstrated *in vitro*. At adequate dosage levels pyrogallol also inhibits this enzyme *in vivo*. In separate studies reported

elsewhere[8] the inhibition of catechol-O-methyl transferase with pyrogallol *in vivo* produced no accumulation of endogenous norepinephrine in the brain or heart of the rat.

Section of Experimental Therapeutics,
National Heart Institute,
Bethesda, Maryland

J. RICHARD CROUT

REFERENCES

1. J. AXELROD, J. K. INSCOE, S. SENOH and B. WITKOP, *Biochim. Biophys. Acta*, **27**, 210 (1958).
2. J. AXELROD and R. TOMCHICK, *J. Biol. Chem.* **233**, 702 (1958).
3. J. AXELROD and M-J. LAROCHE, *Science* **130**, 800 (1959).
4. Z. M. BACQ, L. GOSSELIN, A. DRESSE and J. RENSON, *Science* **130**, 453 (1959).
5. S. UDENFRIEND, C. R. CREVELING, M. OSAKI, J. W. DALY and B. WITKOP, *Arch. Biochem.* **84**, 249 (1959).
6. J. AXELROD, W. ALBERS and C. D. CLEMENTE, *J. Neurochem.* **5**, 68 (1959).
7. S. ARCHER, A. ARNOLD, R. K. KULLNIG and D. W. WYLIE, *Arch. Biochem.* **87**, 153 (1960).
8. J. R. CROUT, C. R. CREVELING and S. UDENFRIEND. In preparation.

Improved preparation of 5-bromo-2′-deoxycytidine

(*Received* 22 *December* 1960)

THE need for a large supply of 5-bromo 2′-deoxycytidine, as well as small amounts of it with suitable isotopic labels, for study in this laboratory as either a chemotherapeutic agent or one to reduce the threshold to radiation injury, has prompted us to re-examine the preparation of this compound. The method described by Frisch and Visser[1] appeared to be impractical for several reasons: first, the method involves the use of a special source of ultraviolet light and, therefore, quartz reaction vessels; second, glacial acetic acid as the solvent for bromination leads to the undesirable acetylation of the deoxyribose moiety, a circumstance which necessitates subsequent deacetylation by methanolic ammonia; third, purification of the product by the use of a column of Amberlite IR-120 (H^+ form) leads to extensive cleavage of 5-bromocytosine from the deoxyribonucleoside.

We have found that bromination of 2′-deoxycytidine can be conveniently and rapidly accomplished in pyridine; the yield of 5-bromo-2′-deoxycytidine is quantitative. When the crude 5-bromo-2′-deoxycytidine hydrobromide was purified by the use of a charcoal column, an 87 per cent yield of free 5-bromo-2′-deoxycytidine was obtained. The deoxyribonucleoside appears to be completely stable on the charcoal column and no formation of 5-bromocytosine was observed even after prolonged standing.

In one experiment, formamide was employed as the solvent for bromination, in a manner similar to that of an unpublished procedure of Markham, referred to by Bessman *et al.*[2], for the preparation of 5-bromo-2′-deoxycytidine triphosphate; the 5-bromo-2′-deoxycytidine thus formed was purified