REFERENCES

1. J. AXELROD, *J. biol. Chem.* **214,** 753 (1955).
2. A. M. ASATOOR, B. R. GALMAN, J. R. JOHNSON and M. D. MILNE, *Br. J. Pharmac. Chemother.* **24,** 293 (1965).
3. L. G. DRING, R. L. SMITH and R. T. WILLIAMS, *Biochem J.* **116,** 425 (1970).
4. HEY, *J. chem. Soc.* 19 (1930).
5. L. G. DRING, R. L. SMITH and R. T. WILLIAMS, *J. Pharm. Pharmac.* **18,** 404 (1966).
6. R. C. FUSON, *Advanced Organic Chemistry*, p. 374. John Wiley, New York (1950).

Biochemical Pharmacology, Vol. 20, pp. 2128–2130. Pergamon Press, 1971. Printed in Great Britain

Different sensitivities of the phosphodiesterases (adenosine-3′,5′-cyclic phosphate 3′-phosphohydrolase) of dog cerebral cortex and erythrocytes to inhibition by synthetic agents and cold

(*Received* 19 *November* 1970; *accepted* 29 *January* 1971)

AFTER the initial report describing the effect of 4-(3,4-dimethoxybenzyl)-2-imidazolidinone as both a lipolytic agent and inhibitor of phosphodiesterase (PD),[1] an even more potent inhibitor of the PD from rat erythrocytes was found in the 3-butoxy-4-methoxybenzyl derivative (Ro 20-1724).[2] In the course of these and other studies, differences were noted in the sensitivity of PD from a variety of tissues to some of these compounds. A systematic examination of the effects of several inhibitors on the PD preparations of the cerebral cortex and erythrocytes of the dog and rat was undertaken.

The assay of PD involved a measurement of the conversion of adenosine (-8-^{3}H)-3′,5′-cyclic phosphate (C-AMP-^{3}H; 4 μM) to AMP in the presence of the enzyme.[1] Incubations were carried out in quadruplicate at 37° or in an ice bath as specified. The erythrocyte PD preparations represent ghost-free hemolysates prepared by mixing 2 ml of washed (three times) erythrocytes with 26 ml of 7 mM phosphate buffer and centrifuging at 15,000 rev/min for 40 min. The cerebral cortex PD preparation was obtained by homogenizing portions of tissue with 5 vol. of 1·15% KCl solution using a glass homogenizer for 0·5 min. The homogenate and washings (5 vol.) were then centrifuged at 105,000 *g* to obtain a relatively particulate-free supernatant. All incubations of the cerebral cortex PD were carried out in the presence of 2×10^{-4} M AMP to trap the AMP-^{3}H and minimize its conversion to adenosine by the 5′-nucleotidase activity of the preparation. This concentration of AMP did not significantly alter the PD activity.

TABLE 1. INHIBITION OF THE PHOSPHODIESTERASE FROM DOG CEREBRAL CORTEX AND DOG AND RAT RED BLOOD CELLS BY THEOPHYLLINE, PAPAVERINE, RO 20-1724, APOMORPHINE AND 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC)

	I_{50} (μM)				
Tissue	Theophylline	Papaverine	Ro 20-1724	DOPAC	Apomorphine
Rat RBC	820	12·0	0·17	> 10,000	170·0
Dog RBC	920	21·0	0·2	> 10,000	220·0
Dog cerebral cortex	600	12·0	275·0	630	15·0

In Table 1 it can be seen that the dog red blood cell (RBC) enzyme behaves very much like that from the rat in that inhibition by Ro 20-1724 > papaverine > apomorphine > theophylline > 3,4-dihydroxyphenylacetic acid (DOPAC). With the dog cerebral cortex, however, the inhibition by papaverine = apomorphine > Ro 20-1724 > DOPAC = theophylline.

The I_{50} values of theophylline for the two RBC preparations are not significantly different, but both are significantly higher ($P < 0·05$) than that for the cerebral cortex. The I_{50} of papaverine with the

dog, but not the rat, RBC enzyme is significantly different ($P < 0.05$) from that with the dog cerebral cortex enzyme. Ro 20-1724 inhibits the two RBC preparations equally, but these I_{50} values are approximately 1000 times smaller than that for the cerebral cortex. With DOPAC and apomorphine, however, the reverse is true. With a concentration of DOPAC as high as 10^{-2} m only 25 per cent inhibition could be obtained with the RBC enzyme, while with the cerebral cortex the I_{50} was similar to that obtained with theophylline.

The recent reports that papaverine is 15–30 times more potent than theophylline with rabbit aorta and uteri PD,[3] or 10 and 23 times more potent with beef heart and coronary artery PD, respectively,[4] in general conforms with the 41·5–68·0 times greater potency obtained above. The activities of the other inhibitors vary over several orders of magnitude with the enzymes from the two types of tissue and suggest that very important differences exist in the PD preparations now being used.

In a recent report, O'Dea *et al.*[5] demonstrated that beef heart PD exhibits a hydrolytic activity at 0° which is more rapid than would have been expected and may be the result of temperature-induced conformational changes. As seen in Fig. 1, the dog RBC activity is reduced much more than that of

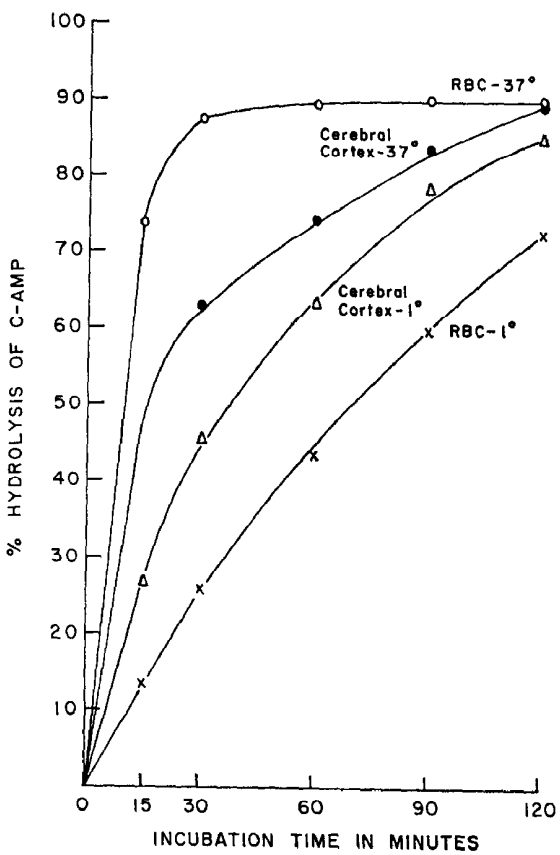

FIG. 1. Effect of temperature reduction on the activity of the phosphodiesterase from dog red blood cells and cerebral cortex.

the cerebral cortex when the temperature is reduced from 37° to 1°. Thus, the RBC enzyme is much more sensitive to both Ro 20-1724 and temperature. Using the 15-min values and van't Hoff's equation for Q_{10}, temperature coefficients of 1·18 and 1·62 were obtained for the cerebral cortex and RBC PD respectively. While these values should not be considered as absolute, they do suggest that for the cerebral cortex PD the Q_{10} approaches 1 over a significant part of the temperature range. For the RBC enzyme, the slight deviation from 2 could be the result of too few experimental points. It is interesting that O'Dea *et al.*[5] report Q_{10} values of 2 from 0° to 10° and of 1 from 20° to 30°. The

decrease in Q_{10} from 2 to 1 suggests some alteration of the enzyme which inhibits the thermochemically induced increase in rate. Such effects are normally observed as a result of heat-induced denaturation of an enzyme and may be expected at about 45–50°. More detailed temperature vs. activity studies are required to obtain a better understanding of this temperature-dependent phenomenon.

The finding of preferential inhibition of cerebral cortex PD by DOPAC and apomorphine was unexpected. Since apomorphine is thought to act on dopamine receptors,[6] and DOPAC is a metabolite of dopamine, one is forced to consider that a tissue specific PD may be an important component of a dopamine receptor.

Finally, it is clear that in order to evaluate the potency of various substances as inhibitors of PD, one must use enzymes prepared from several tissues.

Biochemical Pharmacology Section,
Hoffmann-La Roche, Inc.,
Nutley, N.J. 07110, *U.S.A.*

HERBERT SHEPPARD
GLORIA WIGGAN

REFERENCES

1. C. DALTON, J. B. QUINN, C. R. BURGHARDT and H. SHEPPARD, *J. Pharmac. exp. Ther.* **173,** 270 (1970).
2. H. SHEPPARD and G. WIGGAN, *Molec. Pharmac.* **7,** 111 (1971).
3. L. TRINER, Y. VULLIEMOZ, I. SCHWARTZ and G. G. NAHAS, *Biochem. biophys. Res. Commun.* **40,** 64 (1970).
4. W. R. KUKOVETZ and G. POCH, *Arch. Pharmak.* **267,** 189 (1970).
5. R. F. O'DEA, M. K. HADDOX and N. D. GOLDBERG, *Pharmacologist* **12,** 291 (1970).
6. A. M. ERNST, *Acta Physiol. Pharmacol. neerl.* **15,** 141 (1969).

Biochemical Pharmacology, Vol. 20, pp. 2130–2133. Pergamon Press, 1971. Printed in Great Britain

Effect of polycyclic hydrocarbons *in vitro* on aryl hydrocarbon (benzo[a]pyrene) hydroxylase

(*Received* 18 *September* 1970; *accepted* 4 *December* 1970)

ARYL hydrocarbon (benzo[a]pyrene) hydroxylase is an inducible enzyme system which has been found in many tissues of various mammalian species. It is part of the microsomal enzyme complex which is responsible for the metabolism of a variety of exogenous chemicals which include carcinogens, drugs and pesticides.[1–3] The level of activity of this enzyme system varies with the age, sex, species and environment of the animal.[4,5] This enzyme system hydroxylates benzo[a]pyrene to phenolic derivatives.[6,7] It is also capable of hydroxylating a variety of other polycyclic hydrocarbons.[5,8] The phenolic derivatives are generally relatively weak or inactive as carcinogens. Recent studies, however, have suggested that the hydroxylase enzyme complex may convert polycyclic hydrocarbons to a reactive form.[9–12] The evidence for this enzyme involvement in polycyclic hydrocarbon activation is: (1) the toxic effect of polycyclic hydrocarbons correlates with the level of enzyme in a variety of cells grown in culture;[9] (2) the toxicity of dimethylbenz[a]anthracene and benzo[a]pyrene is inhibited by an inhibitor of the enzyme system, 7,8-benzoflavone;[10] (3) the rat liver microsomal enzyme system catalyzes the formation of covalent complexes of polycyclic hydrocarbons with deoxyribonucleic acid and with protein.[11,12]

In this study we thought it would be of interest to examine the affinity of various carcinogenic and noncarcinogenic polycyclic hydrocarbons for this enzyme system. We have determined the effect of various polycyclic hydrocarbons on benzo[a]pyrene hydroxylation. The effect of the added hydrocarbon may be a measure of its activity as a competitor for the benzo[a]pyrene hydroxylation site on the enzyme.