INTRACELLULAR LOCALIZATION AND CO-FACTOR REQUIREMENT
OF NITROREDUCTASE IN RAT LIVER

RYUICHI KATO* AND TAKAO OSHIMA**

*Department of Pharmacology, National Institute of Hygienic Sciences, Setagaya-ku, Tokyo

**Department of Pharmacology, School of Medicine, Keio University, Shinjuku-ku, Tokyo

Received for publication June 17, 1968

Numerous aromatic nitrocompounds are reduced to aromatic amines by mammalian liver. Fouts and Brodie (1) reported that the nitroreductase was localized in $9,000\times g$ supernatant of liver. Although both NADPH and NADH can serve as electron donors, NADPH was about four times more effective than NADH. It is known that hydroxylamines are formed as intermediate in the reduction of nitrocompounds to aromatic amines (1, 2). However, many works on the reduction of nitrocompounds were carried out by measuring the formation of aromatic amines (1-3).

In the present studies, therefore, the step of nitroreduction was separated into two steps and the intracellular localization and co-factor requirement were investigated.

Male rats of Wistar strain, weighing about 170-200 g were used. The microsomes and supernatant were prepared and the incubation was carried out in atmosphere of nitrogen as described in previous paper (4). *p*-Nitrobenzoic acid (NBA) and *p*-hydroxyaminobenzoic acid (HABA) were used as the substrate. *p*-Hydroxyaminobenzoic acid was synthesized according to the method of Bauer and Rosenthal (5). Determination of *p*-nitrobenzoic acid was carried out spectrophotometrically and *p*-hydroxyaminobenzoic acid was

TABLE 1. Intracellular localization and co-factor requirement of the reduction of *p*-nitrobenzoic acid and *p*-hydroxyaminobenzoic acid by rat liver.

Cell fraction	Co-factor	<i>p</i> -Nitrobenzoic acid		<i>p</i> -Hydroxyaminobenzoic acid
		HABA formed	ABA formed	ABA formed
Microsomes	NADPH	0.86 ± 0.12	0.79 ± 0.05	1.64 ± 0.15
	NADH	0.08 ± 0.02	0.03 ± 0.01	1.14 ± 0.08
Supernatant	NADPH	0.81 ± 0.05	0.08 ± 0.02	0.57 ± 0.04
	NADH	0.76 ± 0.06	0.07 ± 0.01	0.50 ± 0.06
Microsomes + Supernatant	NADPH	1.38 ± 0.14	0.95 ± 0.08	2.32 ± 0.21
	NADH	0.97 ± 0.10	0.50 ± 0.03	1.55 ± 0.11

The activities are expressed by μ mole per g wet weight of liver per 30 minutes and given as the average $\pm$ standard error from 3-4 experiments. The incubation mixture contained the followings in total volumes of 5 ml; 2 ml of tissue preparation equivalent to 500 mg of liver, 0.8 μ mole NADP or NAD, 20 μ mole G-6-P or 40 μ mole alcohol, 25 μ mole $MgCl_2$, 50 μ mole nicotinamide, 5 μ mole *p*-nitrobenzoic acid or *p*-hydroxyaminobenzoic acid, 1.4 ml of 0.2 M phosphate buffer (pH 7.4) and 1.0 ml of 1.15% KCl. G-6-P dehydrogenase (0.5 U) or alcohol dehydrogenase (2.0 U) was added when the microsomes were used. The incubation was run in duplicate at 37°C under nitrogen phase for 30 minutes.

HABA: *p*-hydroxyaminobenzoic acid, ABA: *p*-aminobenzoic acid.

determined by the chelate formation with pentacyanoaquaferroate (6). *p*-Aminobenzoic acid (ABA) was determined by the method of Fouts and Brodie (1).

Table 1 shows that NADPH-linked reduction of *p*-nitrobenzoic acid to *p*-hydroxyaminobenzoic acid is mainly localized in microsomes, whereas NADH-linked nitroreduction is localized in supernatant. The activity of NADPH- and NADH-linked nitroreduction is almost same in supernatant. Moreover, the reduction of *p*-hydroxyaminobenzoic acid is done in both microsomes and supernatant, but the activity is higher in microsomes than that in supernatant.

The NADPH-linked activity is about 50% higher than the NADH-linked activity in microsomes. Thus, the NADH-linked reduction of *p*-nitrobenzoic acid to *p*-aminobenzoic acid is negligible in microsomes, but the NADH-linked reduction in microsomes+supernatant is about 30% of the NADPH-linked activity in accord with results of Fouts and Brodie (1). These results suggested that the first step of the NADH-linked reduction of *p*-nitrobenzoic acid to *p*-aminobenzoic acid is mainly done by supernatant and the second step is mainly mediated by microsomes. The NADPH-linked reduction of *p*-nitrobenzoic acid to *p*-hydroxyaminobenzoic acid is the important step for all over nitroreduction and this step is likely the rate limiting step.

In further experiments it was shown that the NADPH-linked reduction of *p*-nitrobenzoic acid to *p*-hydroxyaminobenzoic acid was inhibited by oxygen and stimulated by FMN or FAD and the microsomal activity was stimulated by phenobarbital. The NADPH-linked nitroreductase of microsomal fraction likely differed from that of supernatant, because the former was stimulated by phenobarbital and inhibited by oxygen, whereas the later was not stimulated by phenobarbital and only partially inhibited by oxygen. The detailed results will be published elsewhere (7).

REFERENCES

- 1) FOUTS, J.R. AND BRODIE, B.B.: *J. Pharmac. exp. Ther.* **119**, 197 (1957); 2) KAMM, J.J. AND GILLETTE, J.R.: *Life Sci.* **2**, 259 (1963); 3) HART, L.G., ADAMSON, R.H., DIXON, R.L. AND FOUTS, J.R.: *J. Pharmac. exp. Ther.* **137**, 103 (1962); 4) KATO, R. AND TAKAHASHI, A.: *Mol. Pharmac.* **4**, 109 (1968); 5) BAUER, H. AND ROSENTHAL, S.M.: *J. Chem. Soc.* **6**, 611 (1944); 6) FEIGLE, F.: *Spot Test in Organic Analysis*, Vol. **2**, p. 121, Elsevier Pub. Comp., Amsterdam (1954); 7) KATO, R., OSHIMA, T. AND TAKANAKA, A.: in preparation

ANTITREMOR ACTION OF A NEW *BETA* RECEPTOR BLOCKING AGENT (I.C.I. 50, 172)

G. ACHARI AND S.P. SINHA

Department of Pharmacology, P. W. Medical College, Patna-4, India

Received for publication June 18, 1968

In a previous communication (1) it was reported that propranolol (Inderal) a *beta* receptor blocking agent abolishes tremor induced by tremorine. A number of structurally similar adrenergic *beta* receptor blocking agents have been synthesized recently and their actions are almost similar qualitatively in that they all block *beta* receptor but they differ quantitatively. A new *beta* receptor blocking agent (I.C.I.50, 172), which does not block all the receptors (2) have been studied in rats to find out the action if tremorine (1,4-dipyrdino-2-butyne) induced tremor and its cholinergic effects are blocked by this new *beta* receptor blocking agent I.C.I. 50, 172.

Methods: Experiments were carried out in albino rats (C.D.R.I., Strain) of both sexes weighing between