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SHORT COMMUNICATIONS

Reduction of Nitrazepam by rat liver

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IN STUDYING the reduction of the 7-nitro group of Nitrazepam (1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin 2-one, "Mogadon Roche") by fractions of rat liver under anaerobic conditions (nitrogen), we made the following observations: purified microsomal fraction, prepared by centrifugation at 105,000 *g* had low potency to reduce Nitrazepam to the corresponding 7-amino derivative. The total reduction of the substrate was only 1·6 per cent per hr per g of liver tissue. On the contrary, the activity of the supernatant isolated at 9000 *g* was seven times higher, yielding 11·1 per cent of reduced Nitrazepam.

When the 9000 *g* fraction was prepared in 0·25 M sucrose instead of using the 1·15 per cent KCl as described by Kato and Gillette,[1] the specific reductive activity increased by 16 per cent per mg of protein per hr. The 9000 *g* fraction of rat liver could be stored, without appreciable loss of activity at 4° for 24 hr according to the finding of Fouts and Brodie[2] for rabbit liver 9000 *g* fraction. The amount of 7-amino derivative produced was linearly proportional to the concentration of 9000 *g* fraction in the incubation mixture. The reaction rate of Nitrazepam reduction was of zero-order, when samples were taken after 15, 30 and 60 min of incubation.

No further metabolization of the 7-amino derivative when added at the concentration of 0·2 μmoles/ml was observed with the 9000 *g* fraction either under aerobic or anaerobic conditions, as it was shown by thin layer chromatography. The Michaelis constant (K_m) of Nitrazepam reduction was $2{\cdot}3 \times 10^{-4}$M, and the V_{max} was $1{\cdot}32 \times 10^{-6}$ moles/hr/g of liver. SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate HCl), a known inhibitor of microsomal enzymes,[3] inhibited the reduction as shown in Table 1.

TABLE 1. EFFECT OF SKF 525A ON NITRAZEPAM REDUCTION

Concn of SKF 525A	Reduction of Nitrazepam Per cent of control
none	100·0
10^{-2}M	39·7
10^{-3}M	62·5
10^{-4}M	69·5
10^{-5}M	81·5

No reduction of Nitrazepam occurred when the preparation was incubated in air instead of under nitrogen. Phenobarbital (50 mg/kg of body weight, twice daily for 4 days) a known inducer of drug metabolism[4] increased the reducing system in liver by 26 per cent (per g of liver tissue) or by 16 per cent (per mg of proteins of 9000 *g* fraction).

METHODS

Male Sprague–Dawley rats (250 g) were used for all experiments. Liver was homogenized in 4 volumes of ice cold (4°) 1·15% KCl or 0·25 M sucrose under standard conditions and centrifuged at 9000 *g* for 20 min at 5°. The microsomal pellet was centrifuged at 105,000 *g* for 60 min and suspended in 1·15% KCl (1 ml of the suspension was equivalent to 400 mg of original liver tissue). The incubation medium contained: 2·5 ml of 9000 *g* (or microsomal) fraction, corresponding to 500 mg (1 g resp.)

of liver tissue and containing 70–80 mg of proteins, NADP (1·5 μmoles) glucose-6-phosphate (50 μmoles), glucose-6-phosphate dehydrogenase (0·5 unit), $MgCl_2$ (25 μmoles), nicotinamide (50 μmoles), potassium phosphate buffer pH 7·0 (280 μmoles) and Nitrazepam (0·2–4·0 μmoles) in a total volume of 5 ml. The samples were incubated in nitrogen atmosphere at 37° while shaking for various periods but usually 1 hr.[1]

The metabolites of Nitrazepam were followed by thin layer chromatography on luminescent silicagel (Kiesegel CAMAG) using the solvent system toluene: acetone: ammonia (50:50:1).[5] Nitrazepam was determined spectrophotometrically[6] at 260 and 310 nm after elution from the thin layer its total recovery was 83% of the incubated quantity. The 7-amino derivative was determined either by direct spectrophotometry at 250 nm or after Bratton–Marshall reaction[5] at 555 nm. Protein determination was performed according to Lowry.[7]

Istituto di Ricerche Farmacologiche I. BARTOŠEK
"M. Negri" E. MUSSINI
Via Eritrea, 62, Milan, Italy S. GARATTINI

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Observations upon the effect of fluoroacetate and pyruvate upon the isolated atria from rat heart

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THE EXPERIMENTS reported here were designed in the hope of confirming with rat heart atria the results of Williamson, Jones and Azzoni;[1] with whole perfused hearts poisoned by fluoroacetate, they found a reversal by addition of pyruvate. This, they interpreted, was due to partial reversal of the fluorocitrate block by increasing amounts of pyruvate, the glycolytic formation of pyruvate being blocked at the phosphofructokinase stage by the increasing citrate formation.[2,3]

Methods and materials. The atria were cut from normal beating hearts of beheaded Sprague–Dawley male rats (190g) fed with a normal balanced diet. They were suspended at 30° in oxygenated Ringer of composition in 1 l., NaCl 9g, KCl 0·42g, $CaCl_2$ 0·24g, $MgCl_2$ 0·005g, $NaHCO_3$ 0·6g, pH 7·3, gas mixture O_2 95% and CO_2 5%. Forty-five min was required for equilibration; it is best to wash three times during this period. For recording the beats a light isotonic lever was used with a load of 0·5g, giving four times magnification and with an ink polyethylene pen.