J. Pharm. Pharmacol., 1965 **17**, 83–88 Received August 27, 1964

Research Papers

Salicylate and aminotransferases

B. J. GOULD* AND M. J. H. SMITH

Enzymic transfer of the amino-group of each of 20 amino-acids to α-oxoglutarate has been studied using lyophilised extracts of rat heart, liver, brain, kidney and skeletal muscle as enzyme source. The glutamate produced was estimated from the carbon dioxide liberated by a bacterial decarboxylase in an autoanalyser. Only asparagine was completely inactive in the transfer. Aminotransferase activity was otherwise unevenly distributed in the tissues. Salicylate (10 mM) was generally inhibitory but it activated the L-tryptophan-α-oxoglutarate aminotransferase.

SALICYLATE has been found to interfere with several important pathways involving the metabolism of glutamate in animal tissue extracts. The drug inhibits the synthesis of glutamine (Messer, 1958), the incorporation of glutamate into protein (Manchester, Randle & Smith, 1958), the conversion of the amino-acid to proline (Bellamy, Huggins & Smith, 1963) and the enzymes responsible for the dehydrogenation and decarboxylation of glutamate (Gould, Huggins & Smith, 1963). An important pathway for glutamate metabolism is controlled by the aminotransferases. These enzymes catalyse the interaction of amino- and α-oxo-acids leading to the interconversion of many amino-acids. Glutamate appears to be a key amino-acid, acting as a donor of amino-groups for the α-oxo-acids corresponding to a number of other amino-acids. Salicylate inhibits alanine and aspartate aminotransferase activities in rat serum and in extracts of rat tissues (Huggins, Smith & Moses, 1961; Yoshida, Metcoff & Kaiser, 1961). These particular enzyme activities have been the most intensively studied but the work of Cammarata & Cohen (1950), Awapara & Seale (1952) and Rowsell (1956) showed that multiple aminotransferase activities, involving glutamate as one of the reactants, occur in mammalian tissues. The purpose of the present work was to study the distribution of these aminotransferases in five rat tissues and to investigate the effect of salicylate on their activities.

Experimental

MATERIALS

γ-Aminobutyric acid, L-ornithine hydrochloride and glutamate decarboxylase (acetone dried powder of *Escherichia coli*) were obtained from the Sigma Chemical Co., St. Louis, Mo., U.S.A. The other L-amino-acids and glycine were obtained from B.D.H. Ltd.; the pyridoxal phosphate from L. Light & Co., Ltd. and all other chemicals were of analytical grade.

From the Arthritis and Rheumatism Council Research Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

* Smith and Nephew Research Fellow.

RAT TISSUE PREPARATIONS

Wistar rats, weighing between 200 and 300 g, maintained on M.R.C. cube diet No. 41, were killed by cervical fracture. The five tissues, liver, kidney, brain, heart and skeletal muscle (quadriceps femoris) from 10 animals, were removed and the bulked specimens of each whole tissue homogenised in twice their weight of ice-cold distilled water, using a Waring blendor followed by a Potter homogeniser. The water homogenates were dialysed for 48 hr at 2° against distilled water and finally freeze-dried. Each batch of freeze-dried preparations sufficed for the investigation of six different aminotransferase activities. Larger samples, prepared from more animals, were not used because it has been shown (Cammarata & Cohen, 1950) that the enzyme activities decrease on storage. Before use the freeze-dried preparations were homogenised in the appropriate media with a Potter homogeniser.

Measurement of aminotransferase activities. All solutions were prepared in 0·1 M potassium phosphate buffer, pH 8·0 Reaction mixtures (0·5 ml) containing the freeze-dried preparation (5 mg) and pyridoxal phosphate (50 μg) were incubated for 15 min at 0° before being added to 0·5 ml of buffer containing 20 μmoles of α-oxoglutarate, 20 μmoles of the appropriate amino-acid and salicylate, when present, to give a final concentration of 10 mM. The mixtures were incubated in stoppered tubes at 37° for 4 hr with constant shaking and the reaction was stopped by placing the tubes in boiling water for 3 min. If necessary, the tubes were stored at −15° before analysis.

The glutamate formed was measured as CO_2 after reaction with glutamate decarboxylase. Each sample was mixed with buffer and the enzyme and the evolved CO_2 automatically estimated. The whole process was carried out with a Technicon Autoanalyser (Technicon Instrument Co., London) which permitted continuous analyses. The method was tested by analysing four samples of each of a series of glutamate solutions of known concentration, ranging from 0·2–10 μmoles/ml. The standard deviation from the mean of the results at each concentration of amino-acid was less than 2% of the total glutamate present. A minimum of four determinations were made for each aminotransferase activity measured with or without salicylate present. The possibility that the constituents of the reaction mixtures may have interfered with the final colour development was excluded by analysing appropriate mixtures in which the various constituents were added to known concentrations of glutamate. Although salicylate inhibits glutamate decarboxylase activity (Gould & others, 1963), the concentrations of the drug in the reaction mixtures after dilution with the various reagents were such that they did not affect the enzyme activity.

Paper chromatographic separations and identifications of the amino- and α-oxo-acids were made and the total α-oxo-acids, total amino-acids and α-oxoglutarate plus glutamate were measured by the methods of Krebs (1950), Rosen (1957) and Meister (1952), before and after the reactions. These procedures were made to ensure that each activity resulted from a true aminotransferase reaction (*cf.* Meister, 1955).

Results

The results (Table 1) show that amino-groups were transferred to α-oxoglutarate from 19 amino-acids in the presence of extracts of one or more of the five rat tissues. These are unequivocal aminotransferase activities because each reaction system satisfies the strict criteria specified by Meister (1955).

L-Glutamine:α-oxoglutarate aminotransferase activity was not unequivocally detected in brain, kidney and skeletal muscle. With brain and kidney extracts, large amounts of glutamate were formed in the reaction mixtures but this was due to the presence of glutaminase which hydrolysed glutamine to glutamate. This reaction obscured any aminotransferase activity which may have been present. Three other L-amino acids studied, lysine, cystine and tyrosine, reacted with the glutamate decarboxylase preparation used for the estimation of the glutamate. It was therefore not possible to determine if aminotransferase activities involving them and α-oxoglutarate occur in the rat tissues. No detectable reaction between asparagine and α-oxoglutarate was found.

Table 1 also shows that 10 mM salicylate, with one exception, significantly inhibits each aminotransferase activity in at least one tissue. There appeared to be no correlation between the degree of inhibition and other factors such as the tissue, the relative activity of the aminotransferase or the chemical type of the amino-acid reacting with the α-oxoglutarate. The exception to this general inhibitory effect of salicylate was the aminotransferase catalysing the reaction between L-tryptophan and the α-oxoglutarate. The enzyme was detected in liver, kidney and heart and in each instance its activity was increased in the presence of salicylate; this effect was statistically significant for the liver and heart.

Discussion

The present results confirm and extend the earlier work of Awapara & Seale (1952) and of Rowsell (1956) in showing that rat tissues possess multiple aminotransferase activities involving glutamate as one of the substrates. Awapara & Seale (1952) used unwashed whole homogenates prepared from eight rat organs and reported the presence of aminotransferase activities involving α-oxoglutarate and the following amino-acids: L-aspartate, L-alanine, L-leucine, L-methionine, L-proline, DL-isoleucine, DL-valine, DL-phenylalanine, DL-threonine and glycine. Rowsell (1956) considered that the use of such homogenates, while providing information about overall amino transfers, was not satisfactory for the recognition of individual aminotransferase reactions because of the presence of endogenous amino- and α-oxo-acids in fresh tissues. This author used washed particles of rat kidney and liver and reported the presence of aminotransferases catalysing the reaction between α-oxoglutarate and the following amino-acids: L-phenylalanine, L-tyrosine, L-tryptophan, L-leucine, L-valine, L-methionine, L-alanine, L-aspartate, DL-norleucine and DL-isoleucine. The present work used lyophilised

TABLE 1. EFFECTS OF 10mM SALICYLATE ON AMINOTRANSFERASE ACTIVITIES IN RAT TISSUES

Control values are the mean of four determinations and are expressed as μ moles of glutamate formed in 4 hr. The salicylate figures are given as percentage inhibitions of the corresponding control value; the differences between the control and salicylate results have been analysed by the *t*-test, Bessel's correction for small samples being used, and values of P are included in brackets. The minimal acceptable level of significance has been taken as P = 0·05.

Amino acid reacting with α-oxoglutarate	Liver		Kidney		Brain		Heart		Skeletal muscle	
	Control	Salicylate	Control	Salicylate	Control	Salicylate	Control	Salicylate	Control	Salicylate
Glycine	0		0		0·3	16 (0·1)	0		0·5	70 (0·05)
L-Alanine	5·1	34 (0·001)	1·6	36 (0·01)	2·5	45 (0·001)	2·1	25 (0·01)	2·9	35 (0·01)
L-Isoleucine	0		0·9	23 (0·05)	1·4	15 (0·2)	2·4	18 (0·01)	0·6	48 (0·05)
L-Leucine	0		1·4	49 (0·05)	1·6	30 (0·1)	1·7	12 (0·05)	2·0	80 (0·001)
L-Valine	0		0		2·9	13 (0·01)	0·6	55 (0·01)	0	
γ-Aminobutyrate	0·6	55 (0·001)	0		1·2	26 (0·05)	0		0	
L-Serine	0		1·8	21 (0·01)	0		0·4	46 (0·01)	0	
L-Threonine	0·3	31 (0·01)	1·4	11 (0·2)	0		0·3	47 (0·05)	0	
L-Aspartate	8·8	0	9·1	4 (0·05)	11·9	7 (0·05)	10·8	6 (0·05)	11·0	0
L-Glutamine	0·8	49 (0·05)	0		0		0·4	47 (0·05)	0	
L-Asparagine	0		0		0		0		0	
L-Ornithine	10·9	10 (0·05)	20·0	0	1·5	10 (0·2)	1·9	8 (0·2)	0	
L-Arginine	2·8	22 (0·01)	0		0		0		0	
L-Cysteine	1·5	30 (0·01)	1·2	19 (0·01)	0·4	23 (0·001)	2·4	51 (0·001)	0·9	27 (0·05)
L-Methionine	0		0		1·0	34 (0·001)	0		0	
L-Phenylalanine	0		0·6	70 (0·001)	1·3	20 (0·1)	1·5	14 (0·3)	1·1	78 (0·01)
L-Tryptophan	1·0	52* (0·01)	1·4	11* (0·3)	0		1·7	38* (0·01)	0	
L-Histidine	0		1·0	10 (0·3)	0		0		0·8	66 (0·05)
L-Proline	0		0·9	6 (0·5)	0		0		0	
L-Hydroxyproline	0		0·7	8 (0·1)	0		0		0	

* Indicates stimulation of activity by salicylate.

preparations from five rat tissues and shows that seventeen L-amino-acids as well as glycine and γ-aminobutyrate, participate in aminotransferase reactions with α-oxoglutarate. There is therefore abundant evidence that aminotransferase reactions involving glutamate, are widely distributed in rat tissues. The multiplicity of these reactions and the relatively high activity of many of the enzymes show that the aminotransferases play an important role in the metabolism of glutamate and of many other amino-acids *in vivo*.

Salicylate possesses a general inhibitory effect on the rat tissue aminotransferases. The salicylate concentration (10 mM) used was high but inhibitions of 50% or more were frequently observed (Table 1) and inhibition could occur *in vivo* with the lower salicylate concentrations (2 to 3 mM) attained and maintained during the therapy of rheumatic disorders in man. It is also relevant that the mechanism of inhibition may involve competition with the α-oxo-acid and amino-acid substrates since this has been shown to be so for the inhibitory action of the drug on alanine and aspartate aminotransferases *in vitro* (Gould, 1964). If this mechanism of inhibition is operative for the other aminotransferases then the degree of inhibition would not depend solely on the salicylate concentration reached in the tissues, but also on the endogenous concentrations of the reactants of the aminotransferase. Salicylate could therefore produce widespread and also differential inhibitory actions on aminotransferase reactions *in vivo* depending on the particular tissue salicylate concentration and on the amounts of α-oxo- and amino-acids present in individual tissues. This should in turn affect the interconversion of many amino-acids and alter the pool sizes of the tissue amino-acids. There is some experimental evidence supporting this hypothesis because Yoshida, Metcoff & Kaiser (1961) and Huggins & Smith (1963) found that the injection of salicylate in the intact rat caused changes in the levels of glutamate and alanine in the liver and brain. A further implication of these effects is that there may be abnormal peptide and protein synthesis due to either a relative deficiency or excess of one or more of the parent amino-acids.

The only exception to the general inhibitory action of salicylate on aminotransferases found during the present work was the enzyme catalysing the reaction between L-tryptophan and α-oxoglutarate. Salicylate increased the activity of this enzyme in the three rat tissues (liver, kidney and heart) where it occurred. The mechanism of this effect of the drug remains to be established.

Acknowledgement. We wish to thank Monsanto Chemicals Ltd. for a grant towards the cost of the work.

References

Awapara, J. & Seale, B. (1952), *J. biol. Chem.*, **194**, 497–502.
Bellamy, A., Huggins, A. K. & Smith, M. J. H. (1963). *J. Pharm. Pharmacol.*, **15**, 559–560.
Cammarata, P. S. & Cohen, P. P. (1950). *J. biol. Chem.*, **187**, 439–452.
Gould, B. J. (1964). Ph.D. Thesis, University of London.

Gould, B. J., Huggins, A. K. & Smith, M. J. H. (1963). *Biochem. J.*, **88**, 346–349.
Huggins, A. K. & Smith, M. J. H. (1963). *Ibid.*, **89**, 112P.
Huggins, A. K., Smith, M. J. H. & Moses, V. (1961). *Ibid.*, **79**, 271–275.
Krebs, H. A. (1950). *Ibid.*, **47**, 605–614.
Manchester, K. L., Randle, P. J. & Smith, G. H. (1958). *Brit. med. J.*, **1**, 1028–1030.
Meister, A. (1952). *J. biol. Chem.*, **197**, 309–317.
Meister, A. (1955). *Advanc. Enzymol.*, **16**, 185–246.
Messer, M. (1958). *Aust. J. exp. Biol. med. Sci.*, **36**, 65–76.
Rosen, H. (1957). *Arch. Biochem.*, **67**, 10–15.
Rowsell, E. V. (1956). *Biochem. J.*, **64**, 235–245.
Yoshida, T., Metcoff, J. & Kaiser, E. (1961). *Amer. J. Dis. Child.*, **102**, 511–512.