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IMPORTANCE OF EXTRAHEPATIC SULPHATE CONJUGATION

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Abstract—A similar trend in the sulphate conjugation of isoprenaline and harmol was observed in the hepatic and three extrahepatic tissues, namely the kidney, small intestine and lung of some experimental animals. All the hepatic and some extrahepatic tissues exhibit this sulphating capability. In fact, some extrahepatic tissues, e.g. monkey lung, kidney and small intestine, mouse kidney and guinea-pig small intestine surpass their respective livers in this sulphate-conjugating reaction. When no or low activity was observed, a consistent pattern was found for both substrates. In general, isoprenaline is a better acceptor than harmol; the greatest difference was obtained with the mouse kidney preparation where an 18-fold difference was attributed partly to the higher sulphotransferase activity for isoprenaline than for harmol. The importance of extrahepatic sulphate conjugation is discussed.

Detoxification of xenobiotics and natural compounds by sulphate conjugation is dependent on enzymes present exclusively in the soluble fraction. This route of disposal could contribute considerably to the overall catabolism of drugs. In this study, two substrates, namely isoprenaline and harmol, were employed in parallel studies to assess the relative contribution by hepatic and extrahepatic tissues to sulphate conjugation.

MATERIALS AND METHODS

Chemicals. Three batches of $Na_2{}^{35}SO_4$, with specific radioactivities of 894, 719 and 815 mCi/mmole, were used in the course of this work. They were purchased from New England Nuclear, Boston, MA, U.S.A.; their radionuclide and radiochemical purity was 99%. L-Isoprenaline bitartrate and ATP (sodium salt, 98% pure) were obtained from Sigma, St. Louis, MO, U.S.A., and harmol-hydrochloride was from Aldrich, WI, U.S.A.

Enzyme preparations and assay conditions. Adult male animals were used unless otherwise specified. The assay conditions for the overall sulphate conjugation from ATP and $Na_2{}^{35}SO_4$, and the sulphotransferase reaction from preformed $PAP^{35}S$ were similar to those described [1], with minor modifications: in the generation of $PAP^{35}S$ from ATP and $Na_2{}^{35}SO_4$, a further 1 hr incubation with 44 mM glucose was included [2], to remove any remaining ATP which is known to inhibit the sulphate conjugation reaction. Secondly, the high-speed supernatant of guinea-pig small intestine was sometimes used as an alternative source for generating PAPS as it was found to possess high sulphate-activating activity [3]. To ensure that the amount of PAPS generated in this manner *in vitro* was not limiting in the subsequent reaction, a preliminary experiment, varying the time of incubation from 0 to 10 min was performed routinely. A 5-min assay time was selected as the sulphotransferase reaction, using such preformed $PAP^{35}S$, proceeded progressively up to 10 min.

RESULTS

The formation of sulphate conjugates of isoprenaline and harmol by liver, kidney, small intestine and lung of various experimental animals is shown in Table 1. It must be emphasized that the data presented in this table were intended to provide qualitative ratios of overall sulphating activity in hepatic and extrahepatic tissues of a number of species of animals, using two different substrates. For comparative purposes, identical experimental conditions have been used. These conditions have previously been shown to be optimum for the sulphation of isoprenaline [1] and harmol [4], and were confirmed in this study when tested on enzyme preparations of monkey lung and guinea-pig small intestine. From Table 1, it can be seen that the hepatic tissues of all the animals are able to form the sulphate conjugates of isoprenaline and harmol from ATP and inorganic sulphate. In most cases, isoprenaline appeared to be a better acceptor than harmol. When little or no activity was detected, the pattern was consistently observed for both substrates. Two animals with considerable sulphating activity in their extrahepatic tissues are the monkey and dog. Of the common laboratory animals, the rat and rabbit showed sulphate conjugation with isoprenaline and harmol almost exclusively in their liver, while in the mouse and guinea-pig, the kidney and small intestine are, respectively, more important than their corresponding livers in this sulphate conjugation reaction.

Comparison between mouse kidney and mouse liver in their overall sulphate conjugation and sulphotransferase reactions

As the mouse kidney is outstanding in the sulphate conjugation of isoprenaline, a systematic study was undertaken to compare its activity with that of the

Table 1. Formation of isoprenaline and harmol sulphates by hepatic and extrahepatic tissues

Species	Liver	Kidney	Small intestine	Lung
Monkey	(78.9)	(146.4)	(116.4)	(342.8)
	78.9	54.9	87.3	236.1
Dog	(404.5)	(447.7)	(92.7)	(225.2)
	341.3	211	42.1	140.7
Mouse*	668 ± 82	2897 ± 443	nil	nil
	97 ± 35	125 ± 29	nil	nil
Guinea-pig	(154.8)	nil	(357.7)	18.5
	163.7	nil	768.3	nil
Rat	(283.3)	nil	nil	nil
	395.9	nil	nil	nil
Rabbit	(64.7)	nil	nil	nil
	74.9	nil	nil	nil

Results are expressed in pmoles isoprenaline ^{35}sulphate or harmol ^{35}sulphate formed/min/mg protein (top and bottom values, respectively) in a 10-min assay, as measured by the three-step (sulphate-activating and sulphotransferase) reaction. Values in parentheses for isoprenaline sulphation are reproduced from ref. [1]. Each figure represents the average of triplicate experiments performed on the same enzyme preparation. These values are intended for qualitative comparison, as opposed to data (*) expressed as means ± S.D., obtained from eight individual animals.

liver in both male and female animals. From Table 2, it can be seen that there is no sex difference in the data with kidney preparations, although there is a tendency of higher hepatic values for female mice. However, except for the two-fold higher overall rate of sulphation of isoprenaline, the difference is not significant. As the enzyme preparations from single animals were insufficient for duplicate assays of the overall and sulphotransferase reactions, a second set of data was obtained with enzyme extracts pooled from five animals. It is obvious that isoprenaline is a better substrate when assayed by either the overall or sulphotransferase reaction. It must be noted that in the sulphotransferase reaction, the amount of PAP^{35}S generated *in vitro* in each set of experiments varied, so that comparison of values could only be made within the same set of data where the concn of active sulphate employed was the same and not limiting.

DISCUSSION

The comparison and competitition between sulphate and glucuronide conjugation of harmol in liver has been studied extensively [5–9], but the extrahepatic contribution to these processes has not been examined. In this investigation, it was demonstrated that some extrahepatic tissues, namely the monkey lung, kidney and small intestine, the mouse kidney and the guinea-pig small intestine are important sulphate conjugating sites. These tissues are, therefore, suitable for studies of extrahepatic conjugation with sulphate, particularly so as they are also efficient in generating PAPS *in vitro* [3].

The common laboratory animals showed differences in their extrahepatic sulphate conjugation. The liver seems to be the only organ in the rat and rabbit capable of forming the sulphate conjugates of isoprenaline and harmol. These two animals would,

Table 2. Formation of isoprenaline sulphate and harmol sulphate by mouse kidney and mouse liver, as measured by (a) the overall reaction and (b) the sulphotransferase reaction

		Overall reaction		Sulphotransferase reaction	
Organ	Sex	Isoprenaline	Harmol	Isoprenaline	Harmol
Kidney	Male	2897 ± 443 (8)	125 ± 29 (8)	—	—
	Male	2236 ± 132	137 ± 13	471 ± 36	51 ± 8
	Female	2371 ± 539	134 ± 27	483 ± 69	56 ± 11
Liver	Male	668 ± 82 (8)	97 ± 35 (8)	—	—
	Male	563 ± 102	103 ± 18	250 ± 29	220 ± 31
	Female	1102 ± 98	138 ± 24	293 ± 21	244 ± 33

Results are expressed as means ± S.D. in pmoles isoprenaline ^{35}sulphate or harmol ^{35}sulphate formed/min/mg protein in a 10-min overall reaction or a 5-min sulphotransferase reaction. Values were obtained from three separate enzyme extracts prepared from five animals of either sex or from the number of individual animals given in parentheses.

therefore, be unsuitable for studies of extrahepatic sulphate conjugation, but they are, however, valuable in sulphation studies which attempt to correlate *in vivo* data with results obtained from isolated hepatocytes or liver perfusion systems. Such a parallelism would not be anticipated in any of the other laboratory animals where the contribution by extrahepatic tissues may be considerable. Of the two animals, the rat appears to be ideal for such studies because of its generally higher sulphating activity, as demonstrated with isoprenaline and harmol and a number of other acceptors [10–12]. The rabbit, on the other hand, is a poor sulphate conjugator and the limited sulphating ability of its bronchial tissues [13], as confirmed in this study, was thought to be responsible for its susceptibility to polycyclic hydrocarbon-induced carcinogenesis [14].

The mouse kidney appeared to be superior to the liver in the sulphate conjugation of isoprenaline and 3-methoxy-4-hydroxyphenylethanol [14]. Under identical experimental conditions the rate of overall sulphation of isoprenaline was about 18 times higher than that of harmol (Table 2), while only a 9-fold difference in their respective sulphotransferase activity was observed. The higher affinity of mouse kidney sulphotransferase for isoprenaline was shown in its apparent K_m of 36 μM compared to 53 μM for harmol; these values were derived from Lineweaver–Burk plots [15]. As the sulphate-activating and sulphotransferase reactions occur sequentially *in vivo*, a situation more closely resembling the overall three-step reaction in the experimental procedure, it would appear that the removal of PAPS by the sulphotransferase is of paramount importance in eliciting an overall higher rate of sulphation.

Man appears to resemble the monkey and dog in that sulphate conjugation occurs in the liver and extrahepatic tissues. This has been reviewed for the biogenic amines and their metabolites [16]. It is possible that in man, and some species of animals, the extrahepatic sites constitute a large sink into which natural and foreign compounds may be distributed, diluted and disposed of by sulphate conjugation. Their actions, singly or combined, may relieve the limitations imposed by the saturation kinetics on the sulphate conjugation reaction observed in the hepatic system.

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