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Sulfation and glucuronidation in the rat in vivo and in vitro

Koster, Hendrik Jan

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SUMMARY

Several compounds possessing a hydroxy group are both sulfated and glucuronidated in vertebrates. The conjugates of some compounds differ in their toxicity. Therefore, it is of interest to understand the mechanisms of competition between the two conjugating reactions. Usually, the fraction of a phenol administered to rats that is sulfated decreases and that is glucuronidated increases when the dose is increased. This limitation of sulfation may be caused by depletion of inorganic sulfate or by saturation of the sulfotransferase with substrate. In the present investigation some factors determining the competition between sulfation and glucuronidation were studied.

A very useful compound in this investigation was 2,6-dichloro-4-nitrophenol (DCNP), a potent inhibitor of sulfation. It proved to be very selective in its inhibition of sulfation; other conjugation and excretion processes remained unaltered (Supplement I). Compounds chemically related to DCNP were also inhibitory but were less effective in vivo (Supplement I, II). When DCNP was administered to the intact animal its action persisted for a long time (more than two days) because it is very slowly eliminated by metabolism and excretion (Supplement III). DCNP is almost completely bound to protein which may explain why its inhibitory action is rapidly reversible in the once-through perfused rat liver: DCNP could be readily washed out of the liver by perfusing with fresh medium containing albumin (Supplement III). When DCNP was injected intravenously in the intact animal the onset of inhibition of harmful sulfation was immediate, which implies that sulfation can be arrested at any desired time (Supplement III). These properties made DCNP very suitable for the study of the sulfation kinetics in vivo (Supplement III) and in the perfused liver (Supplement VI).

Sulfation of various phenols was studied both in vivo and in isolated hepatocytes. When the amount of the sulfate conjugate produced was related to the amount of inorganic sulfate that was present at the start of the experiment (Supplement III, IV), it was found that the relative decrease in sulfation that was observed could not be explained by depletion of inorganic sulfate. However, when high amounts of the sulfate conjugate were produced

after the administration of high doses of the substrate, sulfate availability may be rate-limiting (Supplement IV).

The rate of glucuronidation at steady-state increased more than proportionally with the rate of substrate supply in vivo (Supplement IV) and in the once-through perfused rat liver preparation; this occurred when sulfation became saturated. When sulfation was inhibited by DCNP glucuronidation increased to an extent that compensated completely for the loss of sulfation (Supplement VI). In both cases the total rate of conjugation remained linear with the rate of substrate supply.

We developed a model for cellular uptake of the substrate and its intracellular metabolism (Supplement V). Simulations with this model showed that the unusual kinetics of glucuronidation could be explained by the existence of diffusion-limited metabolism. The apparent aberrancy of glucuronidation is caused by the fact that its rate is related to the extracellular substrate concentration while it should be related to the intracellular substrate concentration. In the latter case its kinetics can be quite normal, i.e. according to Michaelis-Menten. Consequences of such a diffusion barrier on the kinetics of metabolite formation are discussed. It is shown that apparent K_m values of the reactions are dependent on the enzyme-kinetic parameters of competing reactions. From literature data and our own experiments we conclude that although other mechanisms may contribute to the phenomenon of the aberrant kinetics of glucuronidation these kinetics are most fully explained by diffusion-limited metabolism.

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