The Effect of Aspirin (Acetyl Salicylate) on Macromolecule Turnover in Rat Kidney and Liver

It is generally assumed that analgesics, especially salicylate containing mixtures can cause kidney necrosis in animals and humans, particularly when ingested in large doses over long periods[1-3]. However, the biochemical events associated with these changes are far from clear, despite the fact that salicylates have been shown to have a large number of diverse biochemical effects, both in vivo and in vitro[4]. Recently it has been suggested that one effect of salicylate ingestion may be to increase the turnover of proteins in liver[5]. This paper reports studies on the effect of aspirin on macromolecule turnover in rat kidney and liver.

Materials and methods. Male hooded Wistar rats (150–220 g) were used for all experiments. Animals were fed on laboratory pellets, and given either water or aspirin (6 g/l, adjusted to pH 7) to drink. The average consumption of aspirin was 160 mg/day/rat or 800–1000 mg/kg/day. Animals were given a single injection of radioactive compound 3 days after being placed on aspirin or water and sacrificed from 1–21 days thereafter. A single animal was used for each time point and there were 9–11 animals in each group. $^{35}SO_4$ (carrier free) was injected i.p. at 500 μCi/100 g body weight, D-glucosamine-1-^{14}C hydrochloride (58 μCi/μmole) at 4 μCi/100 g body weight and L-leucine-1-^{14}C (62 μCi/μmole) at 4 μCi/100 g body weight. Other methods were as described previously[6,7].

Results and discussion. The effect of aspirin on $^{35}SO_4$-, and ^{14}C-leucine turnover is shown in Tables I and II. It can be seen that this concentration of aspirin in the

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Table I. Effect of sapirin of $^{35}SO_4$ turnover in rat liver and kidney

	$t_{1/2}$ (days)	b	log A^o
Normal kidney mitochondria	3.63	-0.083 ± 0.017	2.97 ± 0.16
Aspirin kidney mitochondria	3.54	-0.085 ± 0.016	3.07 ± 0.15
Normal kidney microsomes	3.54	-0.085 ± 0.016	3.06 ± 0.15
Aspirin kidney microsomes	3.48	-0.086 ± 0.018	3.18 ± 0.17
Normal kidney cytosol	4.09	-0.074 ± 0.033	2.77 ± 0.30
Aspirin kidney cytosol	4.14	-0.073 ± 0.023	2.72 ± 0.21
Normal liver mitochondria	3.80	-0.079 ± 0.033	2.46 ± 0.31
Aspirin liver mitochondria	3.78	-0.080 ± 0.040	2.30 ± 0.36
Normal liver microsomes	3.33	-0.090 ± 0.039	2.18 ± 0.36
Aspirin liver microsomes	4.02	-0.074 ± 0.040	1.89 ± 0.37
Normal liver cytosol	5.93	-0.050 ± 0.047	1.93 ± 0.43
Aspirin liver cytosol	6.39	-0.047 ± 0.043	1.78 ± 0.40

9 rats were injected with $^{35}SO_4$ as described in the text and were killed at intervals from 1–21 days later. The specific activity (expressed as cpm/mg protein) was determined, after correction for radioactive decay, and a least squares fit to a line $A_t = A^o e^{-bt}$ obtained where A_t is specific activity at time t, A^o is initial specific activity obtained by extrapolation, b is the decay constant. All values are $\pm$ 95% confidence limit.

Table II. Effect of aspirin on ^{14}C-leucine turnover in rat liver and kidney

	$t_{1/2}$ (days)	b	log A^o
Normal kidney mitochondria	15.84	-0.019 ± 0.009	2.02 ± 0.09
Aspirin kidney mitochondria	10.68	-0.028 ± 0.005	2.21 ± 0.05
Normal kidney microsomes	7.16	-0.042 ± 0.009	2.22 ± 0.10
Aspirin kidney microsomes	6.09	-0.049 ± 0.012	2.42 ± 0.14
Normal kidney cytosol	6.49	-0.046 ± 0.006	2.32 ± 0.06
Aspirin kidney cytosol	6.08	-0.050 ± 0.011	2.44 ± 0.12
Normal liver mitochondria	8.60	-0.035 ± 0.016	2.14 ± 0.17
Aspirin liver mitochondria	6.08	-0.049 ± 0.018	2.46 ± 0.19
Normal liver microsomes	4.79	-0.063 ± 0.009	2.29 ± 0.09
Aspirin liver microsomes	4.52	-0.067 ± 0.013	2.58 ± 0.14
Normal liver cytosol	5.15	-0.058 ± 0.011	2.27 ± 0.12
Aspirin liver cytosol	4.99	-0.060 ± 0.014	2.49 ± 0.15

9 rats were injected with ^{14}C-leucine as described in the text. Other procedures as Table I.

drinking water has no significant effect on either the turnover rate of $^{35}SO_4$ or the initial specific activity (obtained by extrapolation) of kidney or liver. This result is somewhat surprizing in view of the effects on $^{35}SO_4$ incorporation in vitro previously reported for other tissues[8,9]. The possibility that this dose is inadequate can probably be ruled out because it has been shown that 50% larger doses of aspirin are fatal in 3–4 weeks and also that the dose used in these experiments causes morphological changes in the kidney[10]. Thus it must be concluded that sulphate incorporation and turnover in liver and kidney is rather insensitive to aspirin in vivo.

On the other hand aspirin does have a significant effect on ^{14}C-glucosamine turnover. In both the mitochondrial and microsomal fractions of kidney the degradation rate is decreased and the half life increased from 3.92 to 4.94 days and from 3.62 to 5.11 days respectively ($p < 0.05$). In the case of liver there is also a tendency for the half life to be increased and even though the differences are not significant at the 95% level the results do reinforce the data obtained for kidney. In no case, however, is there any significant effect on the initial specific activity. The results suggest that aspirin may be causing a slower replacement of the mucopolysaccharide backbone, without affecting the rate of sulphate replacement. Other workers[11–13] have suggested that aspirin may have a specific effect on amino sugar metabolism, rather than on other stages of mucopolysaccharide biosynthesis.

Table II shows the effect of aspirin on the turnover of ^{14}C-leucine. In this series of experiments there is a tendency for the half life of the leucine to be decreased, as suggested by MENDELSON et al.[5]. However, the variability of the data is higher than in the case of glucosamine and the differences observed are not statistically significant. Nevertheless it is clear that the tendency is in the opposite direction to that observed for glucosamine.

Throughout this work the apparent decay constants are being measured and the possibility that aspirin may be affecting the extent of reutilization rather than the actual degradation rate must be considered. However the most likely way in which reutilization could be affected would be if aspirin modified the rate of loss of small molecular weight degradation products from the cell. If this were the case one would expect all rates to be modified in the same direction and the fact that this is not observed argues against the effect being on reutilization.

One possible hypothesis which would explain the kidney necrosis caused by salicylate containing mixtures is that these mixtures may interfere with membrane formation by inhibiting mucopolysaccharide synthesis. Because kidney function is particularly dependent on membrane integrity and because there is evidence for a mechanism causing salicylate concentration in the kidney medulla[14] this tissue would be expected to be particularly susceptible to this type of damage. The increased half life of glucosamine reported in this paper supports the idea that salicylate ingestion causes a decrease in synthesis and thus provides support for the hypothesis. The fact that sulphate turnover is not affected seems to indicate that its metabolism is less sensitive to salicylate, in keeping with the results of other workers[11–13].

Résumé. L'ingestion d'aspirine (ester acétylsalicylique) entraîne une diminution significative de la vitesse apparente de la dégradation de la ^{14}C-glucosamine dans le rein, sans modifier la vitesse de dégradation du ^{35}S-sulfate; la vitesse de dégradation de la ^{14}C-leucine semble augmenter. Le foie donne les mêmes résultats.

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The Effect of Drugs on the Release of Endogenous Catecholamines into the Perfusate of Discrete Brain Areas of the Cat in vivo

Many psychoactive drugs alter the turnover of brain catecholamines[1]. However, until recently it has not been possible to directly measure the endogenous catecholamines released from cerebral neurons in vivo. Methods have now become available to determine picogram quantities of dopamine (DA) and noradrenaline (NA)[2]. Using these techniques we have investigated the effects of various drugs on the output of DA and NA into the perfusate of discrete brain areas of the cat in vivo.

Methods. Cats of either sex (2.5–3.5 kg) were initially anaesthetized with ether, a catheter inserted into the femoral vein and tracheotomy performed. All wound and pressure points were repeatedly infiltrated with local anaesthetic. After i.v. injection of gallamine the animals were artificially ventilated and ether was withdrawn. The head of the caudate nucleus or the nucleus ventromedialis of the hypothalamus were perfused with warmed physiological Ringer solution (30 µl/min) by means of a push-pull cannula (2 parallel cannulae welded together, outer diameter of each cannula 0.20 mm) implanted stereotaxically (coordinates: caudate nucleus, A = 16, L = 3.8, H = +4.5; hypothalamus, nucleus ventromedialis, A = 11, L = 1.5, H = −4.5, according to the Atlas of SNIDER and NIEMER[3]). The perfusate of the first 30 min was discarded, thereafter 20-minute samples were collected into chilled centrifuge tubes containing 50 µl of 0.01 *N* perchloric acid. The drugs were injected

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