Divergent Effects of Aspirin and Salicylate on Glucose and Glycogen Metabolism in Human Platelets

Acetyl salicylic acid (aspirin) is one of a number of drugs which has been shown to inhibit the release of ADP from platelets[1-3]. The mechanism of this effect is uncertain; however, it has been shown that aspirin acetylates numerous plasma[4,5] and platelet[6] proteins and it has been suggested that its effect on platelet release is due to this acetylating reaction. In high concentration (55 mM) aspirin has been shown to reduce platelet energy metabolism[7]. The purpose of this investigation was to study the effect of therapeutic levels of aspirin on platelet glycolysis and to compare this with the effects of sodium salicylate which does not have an acetyl grouping (Figure 1) and which is less effective as an inhibitor of ADP release[8].

Suspensions of washed human platelets ($1-2\times10^6$ per mm^3) in a Krebs-Ringer bicarbonate buffer pH 7.4, were prepared from human blood by methods previously described[9]. Glucose was added to the platelet suspension in a ratio of 100 mg per 10^{11} platelets. Stock aspirin solutions, pH 7.4, were prepared immediately before use as previously described[7]. These solutions were tested[10] and found to be free of detectable levels of free salicylate for at least 3 h. Sodium salicylate solutions were also prepared daily in Krebs-Ringer buffer. Glucose uptake and glycogen utilization were measured as previously described[7]. For measurement of glucose uptake and glycogen utilization aliquots of the platelet suspension were incubated for 60 min at 37 °C in unstoppered centrifuge tubes in the presence of increasing concentrations of aspirin or sodium salicylate. At the completion of the incubation time, the tubes were placed in melting ice and agitated for 1 min. A platelet button for glycogen analysis was then prepared by centrifugation at 2500g for 7 min at 4 °C. The supernatant was removed and a portion added to an equal volume of cold 7% perchloric acid and frozen overnight at −20 °C. This was thawed to 4 °C the following day and the precipitated aspirin or salicylic acid removed by centrifugation. Glucose determinations were performed on the supernatant after neutralization with anhydrous sodium bicarbonate by the method of PFLEIDERER[11]. Glucose recoveries were within the range 98–102% and were found to be unaffected by the presence of aspirin or salicylate. Glycogen was extracted from the residual platelet button, as previous described, hydrolyzed to glucose and assayed as above. The extraction of glycogen was found to be unaffected by either aspirin or salicylate.

A randomized Block design analysis was performed on the data from 7 experiments. When a detectable effect of concentration of either aspirin or salicylate on glucose uptake or glycogen utilization was observed DUNNETT's procedure[12] was employed to see at which concentration the effect was significantly different from the control. Tables for determination of p values from DUNNETT's t are not available beyond the 1% level and therefore a significant change can only be shown as $p<0.01$. In several instances however, it is suspected that the true level of significance is even greater.

There was a 17% ($p<0.01$) fall in glucose uptake with the lowest concentration (0.05 mM) of aspirin and this effect appeared to be progressive with increasing concentrations (Figure 2a). At 55 mM aspirin, glucose uptake was inhibited by 54%. There was no significant change in glycogen utilization in concentrations up to 5.0 mM

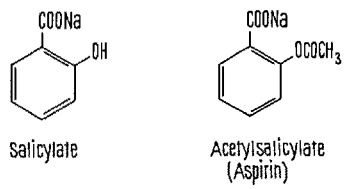

Fig. 1. Structural formulas of sodium salicylate and sodium acetyl salicylate (aspirin).

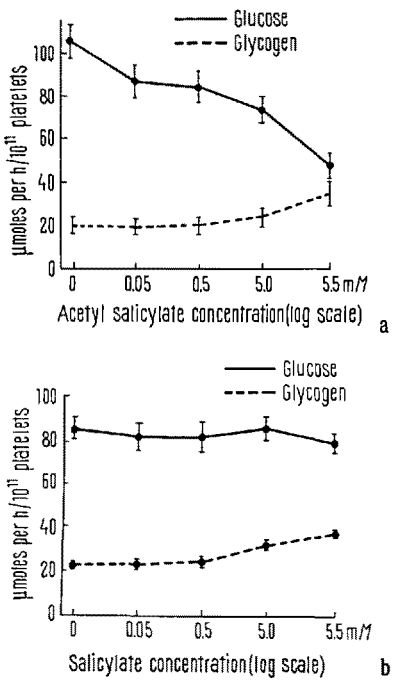

Fig. 2. Effect of (a) aspirin and (b) salicylate on the utilization of glucose and glycogen. Each point of the curve represents the mean value from 7 experiments and the vertical bars denote the extent of ± S.E. Plasma salicylate levels following ingestion of 1.5 g of aspirin are in the range of 0.5 to 1.0 mM[8]. During salicylate poisoning, levels of 5 mM are not uncommon although levels exceeding 10 mM are generally lethal[14].

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($p < 0.05$) but a 75% ($p < 0.01$) increase in glycogen utilization was observed with 55 mM aspirin. In contrast sodium salicylate appeared to have no significant effect on glucose uptake even at 55 mM ($p > 0.05$) but increased glycogen utilization by 44% at 5 mM ($p < 0.01$) and by 69% at 55 mM ($p < 0.01$) (Figure 2b).

The results indicate that aspirin and sodium salicylate have different effects on platelet glucose uptake but they have similar effects on platelet glycogen utilization. However since therapeutic levels of aspirin or salicylate do not normally exceed 0.5 mM it is unlikely that either of these drugs would effect platelet glycogenolysis in vivo. On the other hand the observation that aspirin will reduce glucose utilization at a concentration of 0.05 mM suggests that this effect may occur in vivo following the administration of aspirin in therapeutic doses. This decrease may arise from an inhibitory effect of aspirin on an ATP requiring platelet process such as the energy dependent ADP release which occurs when platelets in washed suspension are exposed to surface stimuli such as the incubating container[13]. Alternatively aspirin may produce this effect by inhibiting glucose uptake either directly or through an effect on glycolysis. This latter effect is unlikely since the rate of glycogen depletion was unchanged and previous studies failed to demonstrate an effect of aspirin on hexokinase activity[7].

The results of these and other studies suggest that aspirin has two types of effects: 1. those arizing from the acetyl grouping which include inhibition of the platelet release reaction[8] and glucose uptake and 2. those arizing from the salicylate moiety which include stimulation of glycogenolysis and uncoupling of oxidative phosphorylation[13,15].

Zusammenfassung. Es wird festgestellt, dass therapeutische Konzentrationen von Aspirin, die die Reaktivität der Blutplättchen hemmen, mit einer signifikanten Herabsetzung der Glukoseverwertung einhergehen. Natriumsalicylat, das keine Acetylgruppe enthält und die Aggregation der Thrombocyten nicht beeinflusst, besitzt dagegen keinen solchen Effekt.

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The Role of Hypercapnia in Acetazolamide Teratogenesis

The carbonic anhydrase inhibitor acetazolamide, when given in large amounts to rats, mice[1] and hamsters[2] during pregnancy, produces malformations almost exclusively of the postaxial portion of the forelimb. The mechanism of teratogenesis is of interest because the lesion is quite specific, and because it apparently involves carbonic anhydrase inhibition[3,4] at an intrauterine site[5,6]. Since a teratogenic dose of acetazolamide raises the tissue pCO_2[7], we tried to imitate the teratogenic effect of acetazolamide with CO_2 enriched air. The possibility of interaction between CO_2 exposure and acetazolamide was also examined. We were aware that HARING[8], using an atmosphere enriched in CO_2 to produce cardiac malformations in rat embryos, reported no limb malformations. But, because the acetazolamide sensitive period and dose response relationship have been thoroughly worked out for the hamster[2], we felt CO_2 exposure during the specific acetazolamide sensitive period was warranted.

Materials and methods. Random-bred, virgin golden hamsters *Cricetus auratus* were mated under direct observation. They were allowed food and water ad libitum. 204 h later, the animals were exposed for 8 h to an increased tension of CO_2 by placing cages in a gas-tight chamber ventilated with CO_2 enriched air. The gas mixture in the chamber was kept at atmospheric pressure, and the concentration of CO_2 in the exhaust was continuously monitored[9].

The plan of treatment is shown in the Table. Some animals were exposed to either 4% or 10% CO_2. Other animals were given the sodium salt of acetazolamide, 600 mg/kg i.p., and then immediately placed in the CO_2 enriched atmosphere. Another group of animals was given a single peritoneal injection of sodium acetazolamide, 600 mg/kg, at 204 h after mating with no additional treatment. All animals were killed approximately 280 h after mating. The fetuses were examined for external malformations, particularly the limbs. The statistical significance of differences between experimental groups was determined by using tables of binomial confidence limits[10]. As used here significant differences refer to confidence limits of 95% or greater.

Results. There were no externally deformed fetuses from the group exposed to 4% CO_2. In the group exposed to 10% CO_2, 2 fetuses with external malformations were found. One had a postaxial deformity of the right forelimb, consisting of a fifth digit perpendicular to the axes of the remaining digits. The other had a preaxial deformity of the right forelimb, consisting of an abnormal angulation of the tip of the second digit.

All external embryonic malformations from the group treated with acetazolamide and 4% CO_2 were confined

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