The effect of aspirin on the protein binding of ascorbic acid

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Tukamoto, Ozeki, Hattori & Ishida (1974) demonstrated the binding of ascorbic acid (AA) to bovine serum albumin (BSA) at 5°C and 20°C by the method of dynamic dialysis developed by Meyer & Guttmann (1968). Using this procedure, AA-BSA binding is shown to occur at 37°C. The presence of acetylsalicylic acid (ASA) is found to reduce this binding. The method of dynamic dialysis involves measurement of the rate of diffusion of AA from a dialysis sac in the presence and absence of BSA. The concentration of free and bound AA in the sac was calculated by measurement of AA diffusion rate. 100 ml aliquots of the Sorenson buffer, at pH 7.38, surrounding the sac were sampled and replenished with fresh buffer at 30 min intervals. AA was analysed by taking 0.5 ml of the sample and adding 4 ml of 0.2 M hydrochloric acid and reading the absorption on an ultra-violet spectrophotometer at 243mμ and compared with a standard curve. Initial sac concentrations of AA, BSA, and ASA were 2×10^{-2} M, 2×10^{-4} M, and 1×10^{-2} M, respectively. All experiments were carried out under nitrogen to prevent the oxidative degradation of the AA. The molecular weight of BSA was taken as 69,000.

Typical results are normally shown in the form of a semi-log plot of total drug concentration against time. In the presence of AA alone, there is a rapid rate of diffusion. When BSA is also present, the rate of diffusion of AA is decreased over time. The slower rate of diffusion indicates that binding of AA with BSA is taking place within the dialysis sac. The degree of separation of these plots is an estimate of the extent of binding of AA to BSA.

The results of these experiments show that AA-BSA binding occurs at 37°C. The introduction of ASA into the sac containing AA and BSA causes the semi-log plot of total drug-concentration against time to approach the line obtained in the absence of BSA. This shows that ASA causes a reduction in the binding of AA to BSA.

By the method of Scatchard (1949) two types of binding sites were isolated for AA binding to BSA. There are ~6 sites per mole of protein of one type. The other sites are non-specific, low-affinity sites of lesser importance.

With ASA present the number of binding sites decreases to ~8 with no change being observed in the non-specific type.

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Synaptosome transmitter release and ATPase activity

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It has been proposed that acetylcholine release at the periphery (Paton, Vizi & Zar, 1971) and in the central nervous system (Vizi, 1972) is triggered by inhibition of sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na^+, K^+-ATPase). A stringent test of this hypothesis should include simultaneous measurements of acetylcholine release and ATPase activity. We have determined the effects of phenytoin, an inhibitor of nerve terminal Na^+, K^+-ATPase, and electrical stimulation on the release of acetylcholine and inorganic phosphate from synaptosomes during the same period to determine any relationship between the two.

Rat cerebral cortex synaptosomes were prepared as described previously (Gilbert & Wyllie, 1976). The synaptosomes were layered on filters and maintained at 37°C in an oxygenated medium (pH 7.4) containing (mM): NaCl (153.5); KCl (5.65); $MgSO_4 . 7H_2O$ (2.8); $CaCl_2 . 6H_2O$ (2.1); $NaHCO_3$ (1.8); glucose (8.3); sucrose (64.3); physostigmine (0.02). Phosphate and/or acetylcholine release were measured over 10 min periods with and without supramaximal electrical stimulation by platinum electrodes (100 Hz, 1 ms, 10 V). Phosphate was determined by the method of Bonting, Simon & Hawkins (1961) and acetylcholine by bioassay using the leech dorsal muscle.

Phenytoin, at a concentration which completely inhibits synaptosome Na^+,K^+-ATPase (5×10^{-5} M), decreased the basal release of phosphate. However, phenytoin did not alter basal acetylcholine release. When electrical pulses were applied acetylcholine release was increased considerably but phosphate release was also increased. Phenytoin prevented the evoked release of acetylcholine and it markedly reduced the evoked release of phosphate.

The question arises as to how much phosphate release is associated with Na^+,K^+-ATPase activity. Experiments involving a number of enzyme inhibitors suggested that approximately 25% of the basal release was limited to Na^+,K^+-ATPase while at least a further 65% resulted from the activities of other ATPases in the synaptosomes.

These results will be discussed in relation to the transmitter release-Na^+,K^+-ATPase hypothesis.

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Effect of reserpine on choline acetyltransferase and high affinity choline uptake in the rat brain

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Regulation of the synthesis of acetylcholine (ACh) in response to nerve impulse flow has been proposed in central (Grewaal & Quastel, 1973) and peripheral (Collier & MacIntosh, 1969) tissues, and adaptational changes affecting neuronal ACh synthesis have been described. Thus choline acetyltransferase (ChA) activities are increased during neuronal activation by drugs (e.g. Mandell & Knapp, 1971; Oesch, 1974). Similarly, changes have been reported in the maximum rate of sodium-dependent high affinity choline uptake (ChU) into brain synaptosomes (Simon, Atweh & Kuhar, 1976). No information was available, however, about the relationship between adaptations of this kind. Reserpine had been shown to decrease ACh concentrations (Beani *et al.*, 1966) and to increase ChA activities in brain (Mandell & Knapp, 1971). We therefore sought adaptations in ChA in

Table 1 Increase of striatal choline acetyltransferase (ChA) and choline uptake (ChU) after treatment of rats with reserpine

Treatment	*ChA activity (nmol/min, 10 mg protein) (1)*	*% of control activity*	*Sodium-dependent uptake of [³H]-choline (pmol/5 min, 10 mg tissue) (2)*	*% of control uptake*
Controls	10.1 ± 0.29	100	3.74 ± 0.076	100
Reserpine 14 h	10.1 ± 0.31	100	4.09 ± 0.18*	109*
18 h	11.9 ± 0.49	118*	4.51 ± 0.11‡	121‡
20 h	12.8 ± 0.86†	127†	4.79 ± 0.40‡	128‡
24 h	10.6 ± 0.41	105	3.73 ± 0.41	100

All ChU values were corrected for sodium-independent (low affinity) uptake by subtracting blanks measured after incubations in a sodium-free medium. [³H]-choline: 0.25 μM throughout.
(1) Mean of duplicate determinations on 6 rats ± s.e. mean; control group, mean of 24 rats.
(2) Mean of 4 determinations on 12 rats ± s.e. mean; control, 24 rats.
Student's 2-tail *t*-tests: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.002$.