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## Inhibition of $Na^+$-$K^+$ dependent ATPase *in vivo* by staphylococcal alpha-hemolysin*

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STAPHYLOCOCCAL alpha-toxin is rapidly lethal for humans[1] and animals[2] with symptoms of central nervous system involvement[3] within the thalamic region.[4] It is suggested[3] that alpha-toxin damages the neuron surface and thus inhibits the polarization–repolarization phase. The results reported in this communication indicate occurrence of serious disturbances in sodium and potassium-dependent ATPase activity. This adds to a better understanding of the mechanism of lethal action of staphylococcal alpha-hemolysin.

*Methods*

Polish lowland breed rabbits weighing 2500–3000 g and Caster guinea pigs weighing 250–350 g of both sexes, were used. Alpha-toxin was purified according to Bernheimer and Schwartz[5] and contained 2 MHD/$\mu$g (minimal hemolytic dose per microgram, rabbit erythrocytes). The toxin sample used killed rabbits within 5–10 min in amount of 30 $\mu$g/kg of body weight, whereas guinea pigs required for the same effect to occur after 20–30 min a dose of 30 $\mu$g/kg.

The animals were injected intravenously with toxin in a dose of 5 $\mu$g/kg (rabbits) and 30 $\mu$g/kg (guinea pigs). 30 min, 2 and 6 hr later, three animals of each species were killed and their organs immediately investigated. Three rabbits received alpha-toxin in a dose of 30 $\mu$g/kg which resulted in a lethal effect within 5–10 min.

From all animals, the brain was removed and samples of liver and kidneys were taken. The material was immediately placed into liquid nitrogen and freeze-dried.

$Na^+$-$K^+$-ATPase activity was measured as described by Bonting[6] and Bonting *et al.*[7] and expressed in milimoles of phosphorus/gram of lyophilized tissue/1 hr of incubation at 37°.

*Results and comment*

Injection of alpha-toxin into rabbits and guinea pigs results in serious disturbances in $Na^+$-$K^+$-ATPase activity, related to the presence or lack of lethal effect (Table 1). In rabbits killed by a toxin dose of 30 $\mu$g/kg sharp inhibition of the investigated enzyme in their brain was regularly demonstrated.

TABLE 1. $Mg^{2+}$ AND $Na^+$-$K^+$-ATPase ACTIVITY IN RABBIT AND GUINEA PIG ORGANS AFTER APPLICATION OF STAPHYLOCOCCAL ALPHA-TOXIN*

| Treatment | Brain | | Liver | | Kidney | |
|---|---|---|---|---|---|---|
| | $Mg^{2+}$ | $Na^+$-$K^+$ | $Mg^{2+}$ | $Na^+$-$K^+$ | $Mg^{2+}$ | $Na^+$-$K^+$ |
| Rabbits (control) | 0·12 | 0·66 | 0·54 | 0·18 | 1.0 | 0·43 |
| Guinea pigs (control) | 0·08 | 0·48 | 0·58 | 0·17 | 0·68 | 0·32 |
| Rabbits (30 $\mu$g/kg) | 0·13 | 0·13 | 0·48 | 0·04 | 0·52 | 0·08 |
| Rabbits (5 $\mu$g/kg) | | | | | | |
| after 30 min | 0·16 | 0·42 | 0·48 | 0·09 | 0·98 | 0·26 |
| after 2 hr | 0·13 | 0·92 | 0·51 | 0·16 | 1·03 | 0·28 |
| after 6 hr | 0·15 | 0·61 | 0·56 | 0·17 | 0·89 | 0·45 |
| Guinea pigs (30 $\mu$g/kg) | | | | | | |
| after 30 min | 0·06 | 0·25 | 0·51 | 0·08 | 0·62 | 0·18 |
| after 2 hr | 0·04 | 0·42 | 0·49 | 0·13 | 0·64 | 0·21 |
| after 6 hr | 0·05 | 0·51 | 0·54 | 0·19 | 0·59 | 0·33 |

* Expressed in mM $P_i$/g/hr (see Methods section). The results are given as a mean value of three determinations.

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The animals surviving alpha-toxin injection, exhibited a decrease of the $Na^+$-$K^+$-ATPase activity by 30–50 per cent within 30 min after injection. The enzyme level was then gradually reaching a normal level.

Marked inhibition of $Na^+$-$K^+$-ATPase activity in rabbits killed by the alpha-toxin and in animals surviving lower doses, suggests occurrence in their organs of an inhibition of sodium and potassium active transport. It cannot be concluded at present whether this is a primary effect. Nevertheless, the observed inhibition of $Na^+$-$K^+$-ATPase activity may produce serious disturbances of sodium-potassium equilibrium in cells and be responsible for brain bioelectric activity changes observed before.[3] Rahal *et al.*[8] have described an inhibition of activite sodium transport using isolated toad bladder. Both observations may suggest importance of active transport inhibition in the mechanism of lethal action of staphylococcal alpha-hemolysin.

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### Monosodium L-glutamate—Inhibition of glucose uptake in brain as a basis for toxicity*

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RECENT reports have implicated monosodium L-glutamate as the causative agent in a variety of pathological and physiological lesions. Oral or parenteral administration of glutamate in amounts in excess of 3 g may be associated with symptoms of burning, facial pressure, chest pain and headache collectively known as the Chinese restaurant syndrome.[1–3] Parenteral administration of glutamate to neonatal mammals has been reported to cause degenerative changes in the inner layer of the retina[4–7] and in the hypothalamus,[8,9] the latter effect probably accounting for the skeletal stunting, obesity and sterility observed.[8,9] There has been controversy over some of these reports,[10,11] but one practical result has been the discontinuation of the practice of adding glutamate to commercial baby foods.

Our own interest in this compound stems from its action as an antagonist of the antimitotic alkaloid, vinblastine.[12,13] There is a competitive relationship between vinblastine and glutamate for entry into human leukocytes,[14] despite their great disparity in chemical structure, and the alkaloid also inhibits

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