Les ensembles enzymatiques participant à la biosynthèse du cholestérol seraient identiques dans les deux classes de membranes, mais avec des densités de distribution propres à chacune d'elles. Si les deux membranes synthétisent chacune pour son compte leur propre cholestérol de structure, le cholestérol destiné au métabolisme général de l'organisme provient essentiellement des membranes endoplasmiques lisses du foie.

Le Tableau II (b) présente l'activité spécifique du cholestérol dans le foie et dans le plasma. Bien que, dès 20 min après l'injection du mévalonate, on observe l'équilibre entre les deux classes microsomiales, au temps 5 min le cholestérol est 2 fois moins actif dans les microsomes lisses que dans les microsomes rugueux; ceci pourrait résulter d'une dilution du cholestérol nouvellement synthétisé par le cholestérol des lipoprotéines non structurales, présentes en plus grande quantité dans les vésicules lisses. L'équilibre observé dès 1 h entre le plasma et le foie résulte des échanges rapides entre le cholestérol plasmatique et le cholestérol synthétisé par les membranes du réseau endoplasmique hépatique.

En conclusion, les données présentées dans cette note sur le réseau endoplasmique de l'hépatocyte adulte montrent que la biosynthèse du cholestérol procède dans les deux fractions lisse et rugueuse selon la même cinétique mais que le réseau lisse, relativement plus riche en enzymes que le réseau rugueux, synthétise la majeure partie du cholestérol hépatique.

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Reçu le 27 novembre, 1967

Biochim. Biophys. Acta, 150 (1968) 326–328

BBA 71016

The rates of action of K^+ and ouabain on the sodium pump in squid axons

It is now known that in nerve and red blood cells there are at least two modes of operation of the sodium pump[1,2]. In one mode internal Na^+ is exchanged for external K^+ and in the other internal Na^+ is exchanged for external Na^+. Both processes are dependent on metabolic energy and are inhibited by the cardiac glycoside ouabain. Na^+–Na^+ exchange is only seen in partially-poisoned squid axons[1] and in the unpoisoned axon 50–90 % of the resting Na^+ efflux is dependent on external K^+. For a proper understanding of the pump mechanism, it is important to know whether

Abbreviations: 10 K(Na)ASW, artificial sea water (composition in mM: NaCl, 460; KCl, 10; $MgCl_2$, 55; $CaCl_2$, 11; $NaHCO_3$, 2.5); 0 K(Na)ASW, contains no KCl; 100 K(Na)ASW, contains in mM: NaCl, 400 and KCl, 100.

this dependence on K^+ represents a rigid linkage between Na^+ efflux and K^+ influx or whether the action of K^+ is less direct. It ought to be possible to distinguish between these two possibilities by measuring the rate at which the Na^+ efflux is reduced after removal of external K^+. If, in the absence of ouabain, the efflux of Na^+ is obligatorily coupled to the influx of K^+, transfer to K^+-free sea water ought to reduce the Na^+ efflux immediately the K^+-free solution reaches the outside of the axon membrane. If K^+-free sea water acts with a lag, this would be evidence for an indirect action of external K^+.

The rates of action of various external solutions have been examined using a special apparatus for effecting rapid solution changes. Giant axons from *Loligo forbesii* were cleaned very carefully to remove most of the adhering small nerve fibres and were loaded with ^{22}Na by micro-injection. Axons were mounted in the outlet arm of a three-way tap. The total dead space was 0.5 ml and the flow rate was 2–3 ml/sec. 2.5 sec was the shortest collection time used. It was possible to stimulate and record electrically from an axon throughout a high-speed run. A measure of the effectiveness of the solution change was that transfer from Na^+ artificial sea water to choline artificial sea water abolished the action potential in 2 sec.

After transfer from 10 K(Na)ASW to 0 K(Na)ASW the efflux was reduced to half in 3–4 sec. Much of this time probably resulted from two processes: (1) the time taken to reduce the K^+ concentration in the immediate vicinity of the axolemma and (2) the time taken to wash out the residual radioactivity from around the axon. An independent estimate of the total time required for these processes was obtained by measuring the rate at which transfer from Na^+ to choline abolished the extra Na^+ efflux during electrical activity. With a tetanization frequency of 100 impulses per sec the time for the Na^+ efflux to fall to half was 3–4 sec. In three experiments these two determinations were compared on the same axon. No significant difference was detected. The similarity between the rates of action of K^+-free sea water on the sodium pump and of Na^+-free sea water on the action potential suggests that K^+-free sea water acts very rapidly. With the time resolution of the present method K^+-free sea water appears to act immediately. If there is a lag, these experiments suggest that it cannot be longer than 1 sec.

The rate of inhibition of the Na^+ efflux by different concentrations of ouabain was measured on axons immersed in 10 K(Na)ASW. In 9 experiments using 10^{-5} M ouabain inhibition was half complete in 37 ± 5 sec (temp. 17–19°). Single determinations in 10^{-3}, 10^{-4} and 10^{-6} M ouabain gave times to half inhibition of 13, 10.5 and 130 sec, respectively. Even in 10^{-3} M ouabain which is a concentration 10^4 greater than that required to produce 100% inhibition the rate of action of ouabain was 3–4 times slower than that of K^+-free sea water. In many experiments there was a brief acceleration of the Na^+ efflux before inhibition set in. The significance of this is not clear.

The most interesting aspect of these experiments was the finding that the rate of action of ouabain depends both on the ionic composition of the external medium and also on the metabolic state of the cell. The importance of ionic composition was investigated by exposing axons to 10^{-5} M ouabain in 10 K(Na)ASW the Na^+ of which had been replaced isosmotically by Li^+, K^+, choline or dextrose. The rates of inhibition in Na^+ and Li^+ were very similar but were almost 10 times faster than in choline or dextrose. The rate in K^+ was intermediate between these extremes. In choline

and dextrose the presence of 10 mM K^+ had no detectable effect on the rates of inhibition. The high-speed apparatus made it possible to apply ouabain for a short period of time and then to switch to a ouabain-free medium. By suitable choice of times it was possible to arrest the process of inhibition at some intermediate stage. In this way it was shown that inhibition of the Na^+ efflux into Na^+ and choline sea waters occurred in a parallel fashion. Thus when the efflux was half inhibited in Na^+ it was also half inhibited in choline and *vice versa*. These observations suggest that in both Na^+ and choline sea waters inhibition is effected by ouabain acting at the same site.

Inhibition by ouabain proved very difficult to reverse. After a 5 min exposure to 10^{-5} M ouabain in 10 K(Na)ASW, washing for 2 h in ouabain-free 10 K(Na)ASW or 1.5 h in ouabain-free 100 K(Na)ASW failed to recover any further ouabain-sensitive Na^+ efflux. However, when 10^{-5} M ouabain was applied in a similar way to an axon which had been poisoned by exposure for 4 h at 18° to 10 K(Na)ASW containing 2 mM cyanide, on first removing the ouabain and then the cyanide a large ouabain-sensitive Na^+ efflux was obtained. In a separate experiment it was shown that the presence of 2 mM cyanide did not itself appreciably interfere with the action of ouabain. The rate of action of ouabain was only reduced in fully poisoned axons. The Na^+–Na^+ exchange which is seen in axons partially-poisoned with alkaline dinitrophenol[1] was rapidly inhibited by ouabain; in a single high-speed experiment with 10^{-5} M ouabain the Na^+ efflux into 0 K(Na)ASW was half inhibited in 15 sec.

One interpretation of these results is that metabolism makes available at the outside of the axon membrane labile groups with which ouabain can combine. This combination is facilitated by the presence of either Na^+ or Li^+ in the external medium. In squid axons the activation of the pump by external K^+ is inhibited[3] by Na^+ and Li^+ and it seems possible that the group with which ouabain normally combines is a part of the transport mechanism which exhibits an affinity for Na^+ and Li^+ and which appears at the outside of the membrane during both Na^+–K^+ exchange and Na^+–Na^+ exchange. Combination of this group with ouabain might block subsequent translocation to the cell interior of both K^+ and Na^+ and thus block Na^+–K^+ exchange and Na^+–Na^+ exchange.

We wish to thank the Director and staff of the Laboratory of the Marine Biological Association, Plymouth, England, for providing excellent research facilities. J.M. was on leave from the Brain Research Unit, University of Brussels.

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Received January 8th, 1968