THE K^+, Na^+ TRANSPORT ATP-ase

The semipermeable plasma membrane of eukaryotic cells serves as a permeability barrier separating the cells from their *surroundings*. The flow of molecules and ions between the cell and its environment is precisely regulated by specific transport systems. In most animal cells the concentration of K^+ is high, and the concentration of Na^+ relative to the external medium is low. These ionic gradients are generated and maintained against passive diffusion by the Na/K pump (K^+, Na^+ transport ATP-ase). The energy needed to keep the pump going is provided by the hydrolysis of ATP. The transport can be inhibited by ouabain - a cardiotonic steroid - that binds to the external surface of the membrane and inhibits the dephosphorylation of the K^+, Na^+ transport ATP-ase. The ATP-ase activity is determined by assaying the amount of liberated inorganic phosphate (P_i) using the Fiske-Subbarow colour reaction.

Solutions:

1.) 0.5 M TRIS HCl buffer pH=7.4
2.) 0.05 M $MgCl_2$
3.) mixture of 1 M NaCl and of 0.2 M KCl solution
4.) 1 M NaCl
5.) 0.2 M KCl
6.) 5 mM Ouabain (Strophantine)
7.) 5 mM ATP
8.) 20 % TCA
9.) 2.5 % Ammonium molybdate
10.) 1 % ascorbic acid
11.) microsomal fraction of grey matter of porcine brain as enzyme source

Preparation of microsomal fraction of porcine brain

Isolating solution: 30 mM TRIS-HCl
0,25 M Saccharose pH= 7.4

Homogenate (20 %) of grey matter of porcine brain was prepared in ***an*** isolating solution. Homogenate was centrifuged at 6,000. RPM for 30 min. at 4ºC (Janetzky K26 centrifuge) and the pellet was discarded. Supernatant was recentrifuged at 40,000 RPM for 60 min at 4ºC (Beckman Ultracentrifuge). The pellet consisting of the microsomal fraction was resuspended in the isolating solution and its protein content was determined with the Biuret method. Microsomal fraction was diluted to a 2 mg/ml of protein concentration in isolating solution.

Experiment

Pipette into test tubes the following ingredients:

Materials/#	**1**	**2**	**3**	**4**	**5**	**6**	**7**
TRIS-HCl ml	0.25	0.25	0.25	0.25	0.25	0.25	0.25
$MgCl_2$ ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NaCl-KCl ml	-	-	0.1	-	0.1	-	-
NaCl ml	-	-	-	-	-	0.1	-
KCl ml	-	-	-	-	-	-	0.1
Oubain ml	-	-	-	0.2	0.2	-	-
H_2O ml	0.3	0.3	0.2	0.1	-	0.2	0.2
ATP ml	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Microsomal suspensions ml	-	0.1	0.1	0.1	0.1	0.1	0.1

Incubate the tubes for 20 minutes at 37 ^{o}C .

Add the followings to the test tubes:

TCA ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Micros. susp.	0.1	-	-	-	-	-	-

Ttransfer the content of the tubes into Eppendorf tubes and centrifuge with 10 000 rpm for 2 min.

Determination of the amount of liberated phosphate (P_i):

Add into 7 numbered test tubes the followings:

Supernatants (from the corresponding Eppendorf tubes)	0.3 ml
H_2O	3,2 ml
Ammonium molybdate	1,0 ml
Ascorbic acid	0.5 ml

After incubation at 37 ^oC for 5 min, cool to room temperature.

Measure the absorption of samples at 720 nm. Water serves as blank. Using the calibration curve calculate the amounts of P_i .
Calculate the percentage of the activity derived from the K^+/Na^+ transport ATP-ase compared to the total ATP-ase activity.