Ouabain Action on Sodium, Chloride and Fluid Transport in Rabbit Jejunum and Ileum

The role of ouabain sensitive Na-K-ATPase in sodium absorption is well established. More recently another ATPase system, also involved in Na active transport, has been found; the latter is not ouabain sensitive[1-4]. Experiments have been performed in order to determine the extent of rabbit jejunum and ileum sensitivity to ouabain.

Materials and methods. The experiments were carried out with ordinary rabbits fed a standard diet and weighing approximately 2–3 kg. The animals were killed by a neck blow and 15–20 cm segment of jejunum was quickly excised 90 cm from pylorus and an analogous segment of distal ileum was cut 20 cm from ileocecal valve. The tissue, rinsed clean of luminal content was put into 37 °C Krebs-Henseleit bicarbonate solution. The composition of the perfusion fluid was: NaCl 118.45 m*M*, $NaHCO_3$ 25 m*M*, KCl 4.75 m*M*, $CaCl_2$ 2.53 m*M*, KH_2PO_4 1.19 m*M*, $MgSO_4$ 1.19 m*M*, glucose 12.9 m*M*, pH 7.4. The fluid was aerated with 95% O_2–5% CO_2. The experiments were performed in winter and spring at a temperature of 34 °C in an air-thermostat. Fluid evaporation was reduced to minimum by large moistening surfaces.

In order to measure the unidirectional Na and Cl influxes (mucosa-serosa), 2 jejunal and ileal adjacent segments were cut along the mesenteric border and put between 2 lucite half chambers of the same volume (7 ml); the apparent surface of the exposed intestinal mucosa was 2 cm^2. The spontaneous transepithelial potential was continuously shortcircuited by an electronic device[5]. At the beginning of each experiment, the mucosal medium was labelled with Na^{22} or Cl^{36} (about 0.5 μCi/ml) and unlabelled Krebs-Henseleit bicarbonate solution was used in the opposite side. Small samples were taken at 15 min interval from the initially unlabelled side. A 40 min equilibration period, necessary to obtain steady fluxes, was followed by 6 15 min periods; after 3 control periods ouabain 10^{-4} *M* or 5×10^{-4} *M* was added to the serosal fluid.

In order to measure the volumetric net transport each experiment was performed as follows: 4 jejunal and 4 ileal adjacent tracts of the same animal, each about 8 cm long, were everted with the aid of a polythene stick and a subsequently fastened at one end to obtain everted sacs: at the opposite end a small tube was ligated, through which serosal fluid was sampled. At the beginning of the experiment, 1 ml Krebs-Henseleit bicarbonate solution was introduced in the serosal side; trace amount of ^{14}C-polyethylenglycol (1 μCi/ml) was also introduced in order to evaluate fluid transport. One jejunal and ileal sac were each filled up with labelled Krebs-Henseleit solution only and used as a control. A second pair contained ouabain 10^{-4} *M* and a third one ouabain 5×10^{-4} *M*. The last couple was tested with DNP 5×10^{-4} *M* added both to the serosal and mucosal side. The everted sacs were then put into a beaker and incubated at 34 °C in Krebs-Henseleit bicarbonate solution gassed with 95% O_2–5% CO_2. At the end of a 40 min equilibration period, a 50 μl sample was taken from the serosal fluid, and followed by an identical sample after a 60 min experimental period in order to evaluate the dilution of the labelled substance during the 60 min period. Each sac was then cut open and dried overnight at 100 °C to obtain the dry weight. From the dry weight and the marker dilution, the fluid transported to the serosal side could be calculated, referred to 1 g dry tissue weight.

All the radioactivity readings were performed with a Tri-Carb liquid scintillation spectrometer Packard (mod. 3003).

Results and discussion. Table I shows that ouabain significantly inhibits sodium influxes both in jejunum and ileum, while it does not influence chloride influxes. It is worth noting that chloride influxes are not dependent on ouabain in both intestinal tracts, although they are both partially metabolically dependent processes[6]. Furthermore the ileal segments are more sensitive to the drug than the jejunal ones, since the maximal inhibition is reached with ouabain 10^{-4} *M* while the jejunal segments show the maximal inhibition with a drug concentration of 5×10^{-4} *M*. Therefore ouabain seems to show a lower activity in jejunum than in ileum[7].

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7 Experiments not reported in this paper show that ouabain 5×10^{-4}*M* does not enhance the ileal Na influx inhibition and also does not modify the Cl influx. Moreover ouabain 10^{-4} *M* affects jejunal Na influx to a less significant extent than 5×10^{-4} *M*.

Table I. Na and Cl influxes ($\varnothing_{m-s}$) in jejunum with ouabain 5×10^{-4}*M* and in ileum with ouabain 10^{-4}*M* in the serosal fluid and percentage variations[a] in the 3 periods following the addition of the glycoside

		Jejunum				Ileum			
		Na		Cl		Na		Cl	
		($\varnothing_{m-s}$)	(%)	($\varnothing_{m-s}$)	(%)	($\varnothing_{m-s}$)	(%)	($\varnothing_{m-s}$)	(%)
Control Periods	1	5.34 ± 0.58		4.25 ± 0.43		6.62 ± 0.65		5.62 ± 0.29	
	2	5.88 ± 0.50		4.90 ± 0.50		7.08 ± 0.92		5.74 ± 0.22	
Quabain Periods	3	4.69 ± 0.39	−18.7 ± 4.7	4.64 ± 0.58	−0.7 ± 3.5	6.47 ± 0.96	− 6.7 ± 6.5	5.66 ± 0.50	−0.4 ± 6.8
	4	4.88 ± 0.44	−16.9 ± 2.7	4.54 ± 0.48	−0.5 ± 1.4	5.61 ± 0.74	−18.5 ± 5.0	5.44 ± 0.36	−5.6 ± 4.8
	5	4.62 ± 0.44	−21.6 ± 2.6 (15)	4.53 ± 0.48	−1.8 ± 2.5 (9)	5.03 ± 0.68	−28.2 ± 2.6 (7)	5.82 ± 0.82	5.2 ± 6.1 (9)

Mean values of the fluxes are referred as μEq $cm^{-2} \times h^{-1}$. Number of experiments in parentheses. [a] Calculated by the formula $(\varnothing_2 - \varnothing_1/\varnothing_1) \times 100$, where $\varnothing_1$ is the second control value and $\varnothing_2$ is each experimental value after ouabain addition. Mean ± SEM.

Table II. Net fluid transport (ΔV) in jejunum and ileum. Effect of various poisoning

	Jejunum		Ileum	
	ΔV (mlg^{-1}h^{-1}) Mean ± SEM	$(\Delta V_2-\Delta V_1/\Delta V_1)\times 100$ Mean ± SEM	ΔV(mlg^{-1}h^{-1}) Mean ± SEM	$(\Delta V_2-\Delta V_1/\Delta V_1)\times 100$ Mean ± SEM
Control	0.629 ± 0.151 (14)		0.800 ± 0.146 (15)	
Ouabain $10^{-4}M$	0.267 ± 0.087 (9)	−54.2 ± 6.0	0.099 ± 0.024 (10)	−82.8 ± 5.1
Ouabain $5\times10^{-4}M$	0.331 ± 0.111 (8)	−53.3 ± 9.5	0.089 ± 0.046 (11)	−92.3 ± 2.0
DNP $5\times10^{-4}M$	0.016 ± 0.014 (7)	−95.5 ± 4.5	0.039 ± 0.014 (7)	−88.0 ± 9.1

Transport values are referred to 1 g dry weight. Number of experiments in parantheses. ΔV_1 represent the control value; ΔV_2 represents the experimental value after drug addition.

In both intestinal tracts net fluid transport is completely inhibited by DNP 5×10^{-4} M while ouabain causes a similar decrease in the ileum. In jejunum, on the contrary, only a 50% inhibition in net fluid transfer is reached (Table II). It seems therefore that net fluid transport in rabbit jejunum is at least partly dependent on a Na-K ATPase system which is insensitive to the drug.

Moreover the results reported on chloride fluxes suggest that the chloride active transport process is, in both intestinal tracts, dependent on another ATPase system which is also not ouabain sensitive.

Riassunto. È stato studiato l'effetto della ouabaina sul trasporto di Na, Cl e fluido in digiuno e ileo isolato di coniglio. Mentre in entrambi i tratti intestinali il flusso mucosa-serosa di Na è inibito dal glicoside, il flusso nello stesso senso di Cl, di cui è stata dimostrata una componente attiva, non viene modificato. Poichè inoltre il trasporto di fluido viene inibito dalla ouabaina in misura diversa in digiuno e in ileo, si suggerisce la presenza di sistemi ATPasici non ouabaino-sensibili accanto alla Na-K ATPasi sensibile alla ouabaina che si ammette essere responsabile del trasporto di Na.

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Ionic Mechanisms Underlying the Depolarization of L-Glutamate on Rat and Human Spinal Neurones in Tissue Culture

Biochemical and electrophysiological studies have provided much evidence that glutamate may function as excitatory transmitter in the mammalian spinal cord (for ref. see[1,2]). Glutamate which is present in high concentrations in the dorsal roots[3,4] has been found to be taken up by a high affinity transport system into synaptosomes and slices of rat spinal cord[5]. Autoradiographic studies on rat and human spinal cord cultures have demonstrated that L-^{3}H-glutamic acid is taken up in neurones as well as in glial cells[6]. Furthermore, it has been shown that microelectrophoretically administered glutamate caused a depolarization of spinal motoneurones of the cat[1,7]. There is, however, little evidence relating to the ionic mechanisms that are responsible for this depolarization. In the present investigation we have used the technique of tissue culture to study ionic mechanisms underlying the depolarizing action of glutamate on rat and human spinal neurones.

Explants from spinal cord of human fetuses (8–18 weeks in utero) and of newborn and fetal rats (18 days in utero) were grown on collagen-coated coverslips for 11–40 days in vitro in the Maximov assembly (for details see[8]).

Intracellular recordings were made with glass microelectrodes (tip diameter $<$ 1 μm) filled with 3 M KCl, 2 M K-citrate or 1 M K-acetate by the method described by TASAKI et al.[9]. The microelectrodes were introduced by micromanipulators from above into the cultures, which were placed in a perfusion chamber[10] mounted on a reverse microscope. For more detailed description see[11,12].

The recording electrode was connected through an Ag-AgCl wire to a cathode follower and potentials were displayed on an oscilloscope and on a rectilinear ink recorder. The temperature of the perfusion solution was

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