The effects of triaminopyrimidine on the short circuit current and transmembrane potentials of the isolated rat visceral yolk sac in vitro[1,2]

P.Y.D. Wong

Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong (Hong Kong), 28 May 1979

Summary. Intracellular potentials in the cells from 17.5-day old rat visceral yolk sacs were measured by a glass microelectrode. When penetrated from the maternal side, the cells have potentials of about 50.2± 1.9 mV (inside negative) which were reduced by increasing the external K^+ concentration and increased by removing Na^+ ions from the bathing fluid. Triaminopyrimidine (TAP) which inhibited Na^+ transport caused a dose-dependent depolarization of the cell membrane. The depolarization was dependent on the presence of extracellular Ca^{2+} ions. It is proposed that TAP may inhibit Na^+ transport by increasing the intracellular concentration of calcium ions.

The rat visceral yolk sac (VYS) has been shown to transport sodium ions actively from the maternal to the fetal side. This may play a role in the formation of the embryonic fluid[3]. Na^+ transport in the VYS occurs against a small transepithelial potential (3.85 mV fetal side positive). It is energy-dependent and inhibited by ouabain. However, unlike Na^+ transport in other epithelia, such as amphibian skins and bladders, it is not affected by the mucosal (maternal) application of amiloride[3]. Recently, triaminopyrimidine (TAP) has been shown to decrease Na^+ transport in leaky epithelia, such as the gall bladder, intestines and choroid plexus[4]. It is of interest to see whether Na^+ transport in the VYS is affected by TAP.

In this work, the intracellular potentials of 17.5-day old rat VYS were measured in an attempt to elucidate the mechanism of sodium transport in this epithelium. The effect of TAP on the SCC and transmembrane potentials was also studied.

Methods. Female Sprague-Dawley rats were placed with males of proven fertility and when sperms were found in vaginal smears in the morning, this was considered to be day 0.5 of gestation. Rats were killed after 17.5 days' gestation. The VYS was isolated as described previously[3]. Preparations were mounted on a perspex platform with the mucosal (maternal) side face - upwards. The platform was placed in a perspex tissue bath (vol. 2 ml) through which Krebs bicarbonate solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 2.56 Mm $CaCl_2$, 1.13 mM $MgSO_4$, 1.17 mM NaH_2PO_4, 11.1 mM glucose) equilibrated with 5% CO_2 in O_2 and prewarmed to 37 °C was pumped at a constant rate of 1 ml/min. The solution was modified in some experiments by augmenting the K^+ concentration or omitting Ca^{2+}. In these cases, corresponding changes in the Na^+ concentration ensured constant osmolarity. When a Na^+-free solution was used, NaCl was substituted by choline chloride (140 mM) and $NaHCO_3$ was omitted. The solution was buffered by Tris (5 mM, pH 7.4) and gassed with pure O_2. Transmembrane potentials were measured by glass microelectrodes filled with 1.5 M K citrate (tip potential < 5 mV, resistance 50–100 MΩ) via the M-701 microprobe system (W-P Instruments Inc). The potentials were recorded with respect to the indifferent electrode placed in the bath. They were displaced on an oscilloscope (Solantron, Schlumberger) and pen recorder (Devices).

Transepithelial Na^+ transport was measured by the short circuit current (SCC) technique described previously[3]. The

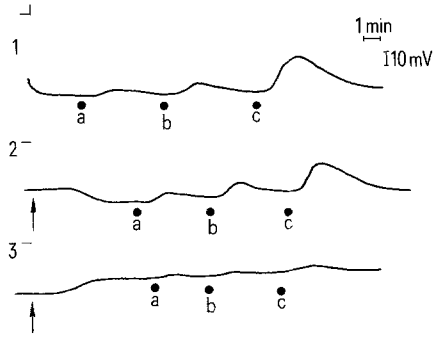

Fig. 2. Tracing of membrane potential recordings from a single cell in 17.5-day old rat visceral yolk sac. A deflection downwards represents an increased negativity of the microelectrode. The sudden jump in potential seen in the left part of 1 corresponds in time to the insertion of the microelectrode into the cell. The dots indicate injection of TAP to the bath to produce a final concentration of (a) 2.5, (b) 5 and (c) 10 $mM l^{-1}$ respectively. Panel 2 is continuous with 1. At the arrow, the superfusion fluid was changed to a Na^+-free solution. The dots indicated the application of TAP as in 1. Panel 3 is continuous with 2 but recorded 5 min later, when the superfusion fluid was returned to normal Krebs solution. At the arrow, the tissue was superfused with a Ca^{2+}-free solution. TAP was applied to the bath as above.

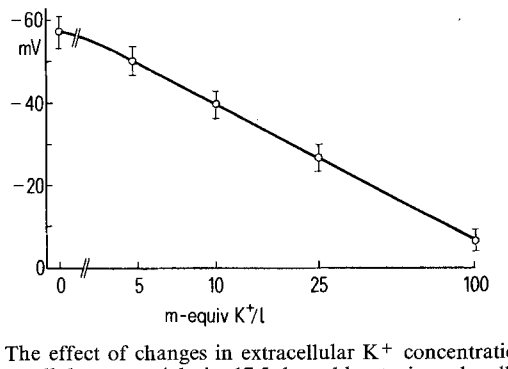

Fig. 1. The effect of changes in extracellular K^+ concentration on the intracellular potentials in 17.5-day old rat visceral yolk sac. Each point shows the mean± SE of more than 30 impalements in 5 different rats.

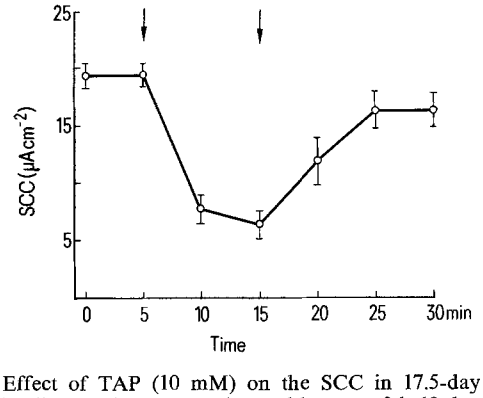

Fig. 3. Effect of TAP (10 mM) on the SCC in 17.5-day old rat visceral yolk sac. The sac was clamped between 2 half-chambers as described previously[3]. TAP was added to both sides of the sac. Each point shows the mean± SE of 5 experiments.

SCC in the rat VYS has been shown to be contributed by a net transepithelial transport of sodium.

Results. The rat VYS consists of a layer of epithelial cells resting on a basement membrane[5]. Penetration of the serosal (fetal) membrane with the microelectrode was found to be difficult and this resulted in low potentials. In contrast, mucosal (maternal) penetration gave larger potentials which were stable for over 30 min. The mean potential of 17.5-day old VYS was found to be 50.2 ± 1.9 mV (inside negative) (63 impalements from 6 rats).

The ionic basis of the transmembrane potentials in the 17.5-day old VYS was investigated by altering the ionic concentrations of the superfusing fluid. Increasing $[K^+]_0$ was found to depolarise the membrane (figure 1). A 10-fold change in $[K^+]_0$ resulted in a 30 mV depolarization. When Na^+ was removed from the external medium there was a hyperpolarization of about 10 mV (control 50.2 ± 2.4; Na^+-free 60.4 ± 2.6, $n = 20$; $P < 0.02$) (figure 2). Removal of calcium depolarized the membrane from 51.3 ± 2.2 to 42.4 ± 2.3 mV ($n = 15$, $P < 0.02$) (figure 2). The transmembrane potential was not affected by amiloride, but was by triaminopyramidine (TAP). Application of TAP to the bath caused a dose dependent depolarization which was still observed in the Na^+ free condition. This depolarization, however, was markedly reduced when Ca^{2+} was omitted from the solution (figure 2). In some experiments, transepithelial sodium transport was measured by the SCC. TAP (10 mM) added to both sides was found to cause a fall in the SCC (figure 3).

Discussion. These results showed that the mucosal (maternal) membrane of the rat VYS is primarily controlled by K_i/K_0 since the transmembrane potentials varied approximately with the log of the external K^+ over a wide range. Removal of sodium from the external solution raised the potentials by 10 mV; sodium ions seem to contribute to the genesis of the potentials. The hyperpolarization effect may suggest that the mucosal membrane of the VYS has a high permeability to Na^+ ions, a finding consistent with a sodium transporting epithelium. Application of amiloride had no effect on the transmembrane potentials. This drug was found to have no effect on Na^+ transport in the VYS[2]. Triaminopyrimidine, a drug known to affect sodium transport in other leaky epithelia inhibited the SCC in the VYS and depolarized the mucosal (maternal) membrane. The depolarizing effect of TAP on the membrane potential was markedly reduced in the absence of external Ca^{2+} ions. It is conceivable that the membrane effect of TAP is to increase the Ca^{2+} influx into the cells. In this context, Grinstein and Erlij[6] has recently provided evidence that intracellular Ca^{2+} regulates Na^+ transport in the frog skin. An increase in the intracellular Ca^{2+} concentration is associated with a fall in the rate of transepithelial Na^+ transport in this tissue. A similar mechanism may also operate in the VYS. It is proposed that the reduced epithelial Na^+ permeability observed with TAP may be due to an increase in the cytoplasmic calcium, although it is not known whether a rise in the cellular calcium affects the intercellular pathway through which ion translocation is believed to occur in leaky epithelia[7].

1 This work was supported by the University of Hong Kong (grant number 335.034.5105).
2 Acknowledgment. Triaminopyrimidine was synthesized by Dr. Barbara Roth of the Wellcome Research Laboratories.
3 S.T.H. Chan and P.Y.D. Wong, J. Physiol. Lond. *279*, 385 (1978).
4 J.H. Moreno, J. gen. Physiol. *66*, 97 (1975).
5 F. Beck, J.B. Lloyd and A. Griffiths, J. Anat. *101*, 461 (1967).
6 S. Grinstein and D. Erlij, Proc. R. Soc. Lond. *202*, 353 (1978).
7 E. Frömter and J. Diamond, Nature New Biol. *235*, 9 (1972).

D(−)Lactic acid – a physiological isomer in the rat

D. Giesecke, Perdita v. Wallenberg and Angelika Fabritius

Institut für Physiologie, Physiologische Chemie und Ernährungsphysiologie der Universität München, Veterinärstrasse 13, D-8000 München 22 (Federal Republic of Germany), 2 July 1979

Summary. D(−)Lactate is produced in significant amounts together with L(+)lactate in the stomach of normal experimental rats. It is absorbed into the blood and constitutes a physiological isomer in this animal species.

Since lactic acid produced in animal metabolism has been proved to be the L(+)isomer[1] the production of D(−)lactate was considered unlikely. Lang[2] has mentioned that some D-lactate is produced in animal metabolism by glyoxalase, the substrate of which is methylglyoxal. As no literature was cited this may have referred to invertebrates, since in vertebrates methylglyoxal is directly converted into pyruvate[3]. In ruminants D-lactate produced by rumen microbes can cause severe D-lactic acidosis[4] which emphasizes the unphysiological nature of the D-isomer.

However, rats fed high levels of dietary DL-lactate excreted in the urine only 1–3% of the D-isomer consumed, and ^{14}C-labelled D-lactate was equally well metabolized by rats fed normal or lactate diets[5]. Thus, rats seemed to be adapted to D-lactate irrespective of dietary intake. In the present work a physiological production of D-lactate in the rat was taken into consideration. The extended non-secretory part of the stomach obviously used for food storage[6] appeared to be a likely organ for D-lactate production. In order to prove this hypothesis, 12 Sprague-Dawley rats of both sexes fed ad libitum a commercial pelleted diet (Altromin No. 1324 with about 50% carbohydrate and 19% crude protein) were killed at 11.00 h. Whole stomach contents was collected and mixed immediately with 0.6 M $HClO_4$. After centrifugation (15,000 × g, 10 min) L- and D-lactate were determined

Table 1. Lactate isomers in the stomach contents of rats

Number of animals		12
Body weight (g)		203 ± 16*
Weight of stomach contents (g)		5.94 ± 1.75*
Amount of lactate present (mmoles)	L(+)	0.43 ± 0.22*
	D(−)	0.30 ± 0.10*
Concentration of lactate (mmoles/kg)	L(+)	68.7 ± 15.7*
	D(−)	50.6 ± 6.9*
Ratio of isomers L:D		1 : 0.77

* Results are means ± SD.