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].

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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.

using specific enzymes (EC 1.1.1.27 and EC 1.1.1.28, Boehringer, Mannheim). Blood samples were treated in the same way.

The results given in table 1 indicate that both L(+) and D(−)lactate were present in stomach contents at the remarkable mean concentrations of 6.18 and 4.56 g/l. The variability between individuals was higher for L than for D with coefficients of variation of 22.8% and 13.6%. The feeding ad libitum was assumed to be the main reason for this variability. The ratio of isomers L:D averaged close to 1:0.8 but varied from 1:0.5 to 1.0:1.1. It was thus apparent that D-lactate is normally produced in the stomach of rats, but for induction of metabolic (enzymatic) adaptation the absorption of the isomer as such would be necessary. Blood samples obtained at random from the hearts of 5 of the above animals contained 46±24 µmoles D-lactate/l, which suggested absorption.

Further evidence was provided by a different experiment in which rats were fed a test-meal containing DL-lactate. Groups of 6 rats were sacrificed after 30–180 min and D-lactate concentrations in blood samples from the vena portae and aorta were compared to similar samples from 10 untreated rats (0 min). The mean animal weight was 248±2 g and the mean intake of D-lactate 101±2 µmoles. The results summarized in table 2 show D-lactate already present in zerotime samples, which is in agreement with the observations given above. Within 30 min after lactate feeding portal D-lactate increased 6-fold, indicating rapid absorption. The portal peak level was reached at 90 min with about 10-fold concentration over normal, and after 180 min most of the stomach lactate appeared to have been eliminated. The comparison with the concentration of D-lactate in aortal blood indicates metabolism of this isomer. It is worth noting that within 30–120 min after lactate intake the D-lactate concentration in portal blood was about 2–3 times higher than in aortal blood. Thus the metabolic rate seemed to keep pace with absorption.

In conclusion, D-lactate appears to be a physiological isomer in the rat. This general result would explain the metabolic adaptation of rats to D-lactate reported previously[5], and may also indicate - at least for the rat - the physiological role of D(−)2-hydroxy acid dehydrogenase (E.C. 1.1.99.6) which was questioned up to now[7]. Furthermore, our data suggest that gastrointestinal production of D-lactate in monogastric mammals is more likely than intermediary generation of this isomer by glyoxalase activity[2]. Similar conditions to those shown for the rat may also exist in other monogastric mammals with a nonsecretory gastric (or pregastric) compartment.

Table 2. Concentrations of D(−) lactic acid in the blood after a test-meal

Time after test-meal (min)	Blood concentration of D(−) lactate (mmoles/l)* Vena portae	Aorta	Difference
0	0.31±0.13	0.27±0.14	0.04
30	1.86±1.03	0.64±0.17	1.22
60	1.57±0.60	0.53±0.27	1.04
90	2.94±1.62	1.19±0.33	1.75
120	1.40±0.60	0.76±0.20	0.64
180	0.77±0.47	0.71±0.51	0.06

* Results are means ± SD.

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Voltage clamp method on single cardiac cells from adult rat heart

A.I. Undrovinas, A.V. Yushmanova, S. Hering and L.V. Rosenshtraukh[1]

Laboratory of Cardiac Electrophysiology, the All-Union Cardiology Research Center AMS, Petroverigskiy Lane 10, Moscow 101837 (USSR), 4 May 1979

Summary. Voltage clamp experiments on isolated cardiac cells from adult rat hearts were carried out using an intracellular dialysis method. The fast inward current was recorded. Tetrodotoxin (TTX) at a concentration of 2.5×10^{-7} g/ml blocked this current to 30% of its initial value. Inward maximal fast current density was calculated to be 0.14–0.7 mA/cm^2.

The multicellular structure of myocardial preparations used in voltage clamp experiments is the main difficulty for qualitative analysis of membrane ionic currents[2-4]. Therefore, the purpose of the present paper was to record ionic currents on single cardiac cells using the intracellular dialysis technique[5].

Materials and methods. The experiments were performed on single myocytes from adult rat hearts isolated by a method described previously[6]. Disaggregation of the heart tissue was carried out by treatment with collagenase at a concentration of 0.8 mg/ml (type 1, Sigma) and Ca^{2+} free buffer at 37 °C by Langendorff perfusion. Cell suspensions were kept at room temperature in 5 ml buffer (pH 7.4) containing (in mM): 118 NaCl, 4.8 KCl, 0.9 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1 mg/ml bovine amniotic fluid (Serva), gently bubbled with 95% O_2–5% CO_2. This solution was designated in experiments as 'extracellular' solution. Cardiac cells were viable for 5–9 h. In the voltage clamp experiments we used the method of intracellular dialysis used for the neuroblastoma cells[5]. V-shaped plastic tubes with a conical pore on the tip were made for cardiac cell dialysis. The dimensions of the pore were: outer diameter 20–30 µm, inner diameter 10–15 µm. The pore was covered with an adhesive material consisting of 40% vaselin oil and 60% parafilm (USA) prepared by heating for about 1 h in a water bath. The tube was perfused with 'intracellular' solution (150 mM tris-PO_4, pH 7.3), reference and current passing electrodes (Ag-AgCl/3 M KCl) were placed in the output branch of the tube. The tip of the tube was immersed in the experimental chamber perfused with the