Intramuscular injections of 1,2-propanediol can stimulate rumen papillary development of milk-fed kids

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Summary. By using the scanning electron microscope technique, it was demonstrated that intramuscular injections of 1,2-propanediol (2.5 ml per day for 10 days) can stimulate rumen papillary development of milk-fed kids.

Postnatal growth of the ruminant's forestomach (rumen, reticulum and omasum) occurs mostly after active fermentation of solid food is established in the rumen. Experiments[1-4] have suggested that volatile fatty acids, especially butyric and propionic acid, produced by microbial fermentation in the rumen become the chemical stimulants for rumen mucosal growth. In previous work to find out whether there is a stimulant other than butyrate or propionate, it was found that intraruminal or intra-abomasal administration of 1,2-propanediol (propylene glycol) can elicit rumen papillary development in kids and lambs[5,6] and increase the labeling index of rumen basal cells[7]. The object of this work was to examine the effects of intra-abomasal or i.m. administration of 1,2-propanediol on rumen papillary development of milk-fed kids by using the scanning electron microscope technique.

Methods. Japanese native meat-type kids of 3 days of age were placed in metal cages and taught to drink milk from nipple bottles. During the experimental periods 300 ml per day of reconstituted milk containing 20% of fat- and water-soluble vitamin-fortified milk powder were fed to each animal in equal parts at 09.00 h and 16.00 h. No other food was fed. In experiment 1, 30 ml of aqueous solution containing 15% 1,2-propanediol was mixed with 150 ml of warmed milk and nipple-fed twice daily for the last 2 weeks before the animals reached 5-7 weeks of age. Control animals received water instead of the propanediol solution. In experiment 2, 5 ml of the mixture of 1,2-propanediol and 0.9% saline at the ratio of 1:1 were injected i.m. twice daily for last 10 days before 3-5 weeks of age. Control animals did not receive the injection. Experiment 3 was the same as experiment 2 except that 5 ml of the mixture was injected once daily until 3 weeks of age. After the treatment periods, we slaughtered the animals after anesthetizing with ethyl ether, and manually separated the rumen mucosal layer from the muscular layer and measured their weights. Rumen papillae collected from the anterior part of the rumen were fixed in 10% neutral formalin overnight and post-fixed in 1% osmium tetroxide routinely. After hydration through a series of graded ethanols and an isoamyl acetate bath, the papillae were dried in liquid CO_2 by the critical point drying method. They were coated with gold and observed by a FE-type scanning electron microscope (Hitachi S-700). Papillary length and width (5-9 papillae per animal) were measured at the middle portion of a papilla with the photomicrographs taken at magnification of $\times 80$-150.

Results and discussion. As shown in the table, in experiment 1 the ratio of rumen mucosa to muscle layer and papillary length and width of the animals given 1,2-propanediol intra-abomasally were significantly higher than those of the control. This result confirmed our previous observation[5]. In experiment 2, apparently the animals injected 1,2-propanediol showed more extensive papillary development than the control, but no significant differences were obtained because of small numbers of the animals used and large variation of the slaughter ages. In experiment 3, the ratio of rumen mucosa to muscle layer and the papillary length of the animals injected with 1,2-propanediol were significantly higher than those of the control. The gas chromatographic analysis of plasma concentrations of 1,2-propanediol in experiment 2 showed that 1,2-propanediol injected at 09.00 h and 16.00 h remained in the circulating blood throughout a whole day. This observation and the previous work[7] using radioactive 1,2-propanediol suggest that 1,2-propanediol is slowly metabolized in the body and that the compound may have a special ability to penetrate into cell boundaries and cell membranes of rumen epithelium tissue.

Although 1,2-propanediol is a glucogenic substance to be metabolized via lactic acid and used as a vehicule in some pharmacological preparations[8], the administration of 1,2-propanediol has been reported to have a keratolytic or allergic effect on human skin[9,10], a toxic effect on mouse bladder epithelium[11], a cardiovascular effect on calves[12], an antiarrhythmic effect on rabbits[13], a physical distortion effect on hepatic endoplasmic reticulum[14], an induction effect on hepatic enzymes of rats[15], an increasing effect on hepatic cholesterol content of rats[16] and a perturbation effect on erythrocytes of rats[17]. These facts suggest that

Effects of intra-abomasal or intramuscular administration of 1,2-propanediol on rumen papillary development of milk-fed kids

Experiment	Treatment	No. of animals	Final body weight (kg)	Rumen weight Mucosa (g)	Muscle (g)	Ratio of mucosa to muscle	Rumen papillae Length (mm)	Width (mm)
1	Propanediol	5	2.94±0.18	9.0±1.4	8.9±1.5	1.03±0.23	0.85±0.14	0.33±0.07
	Control	5	2.83±0.17	6.4±0.7	8.6±0.8	0.74±0.06	0.60±0.11	0.21±0.03
			NS	$p<0.01$	NS	$p<0.05$	$p<0.05$	$p<0.01$
2	Propanediol	3	2.79±0.32	7.0±1.4	7.2±1.3	0.97±0.09	0.74±0.25	0.20±0.03
	Control	2	2.43±0.11	5.1±1.1	6.6±0.9	0.78±0.06	0.60±0.03	0.18±0.01
			NS	NS	NS	$p<0.10$	NS	NS
3	Propanediol	6	2.32±0.18	5.3±1.0	5.2±0.7	1.01±0.08	0.61±0.19	0.19±0.03
	Control	6	2.36±0.38	4.5±0.8	5.7±0.9	0.80±0.07	0.37±0.13	0.16±0.01
			NS	NS	NS	$p<0.01$	$p<0.05$	$p<0.10$

Data are shown as mean±SD. P, Level of significance of difference between the propanediol-treated group and the control. NS, not significant. In experiment 1, 9 ml per day of 1,2-propanediol were intra-abomasally administered for last 2 weeks until 5-7 weeks of age. In experiments 2 and 3, 5 and 2.5 ml per day of 1,2-propanediol were injected i.m. for last 10 days until 3-5 and 3 weeks of age, respectively. These kids were of the same breed as those used in our previous experiments[5,7]. The average daily gain of 13 control animals and 14 treated animals in experiments 1-3 were 45±11 (SD) and 43±12 g, respectively, in the above administration periods.

either 1,2-propanediol or its metabolite may be a biologically active agent in vivo. The stimulating effect of 1,2-propanediol on rumen mucosal growth may be more specific than that of volatile fatty acids, since the latter compounds are effective only through the intraruminal administration route[5].

In the case of the effect on rumen papillary development, our present hypothesis is that 1,2-propanediol or its metabolite may be directly involved in the process of cell proliferation after the compound reaches the region of rumen basal cells through the bloodstream. Further studies will be required to reveal the mechanism of this phenomenon.

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The control of contraction activation by the membrane potential[1]

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Summary. Force measurements under voltage-clamp control were performed in short toe muscles of the frog. The results show a) that the activation of force development and its repriming process can at least qualitatively be correlated to charge movement kinetics; b) that caffeine improves EC coupling by potentiating the potential dependent activation process.

Force development in skeletal muscle fibres of the frog is initiated in a narrow potential range. It starts when fibres are depolarized to -50 mV and reaches its maximum at -35 mV[2]. The increase in oxygen consumption at more negative potentials than -50 mV[3] (Solandt effect) and the further increase in Ca release by depolarizing pulses beyond -35 mV[4] suggest, however, that the control mechanism for Ca release operates in a broader potential range. The results cited correspond qualitatively to the potential dependence of intramembrane charge movement in the transverse (T) tubuli[5,6], supposed to initiate force activation by causing in some way the release of Ca from the terminal cisternae of the sarcoplasmic reticulum[7]. Our present voltage-clamp analysis is consistent with the view that a certain amount of charge activation is needed to initiate force[8] and that the latter saturates before all charges are activated.

The experiments were performed with single fibres in small bundles dissected from the M. lumbricalis digiti IV of the hind limb of *Rana temporaria*. A 2 micro-electrode voltage clamp technique was employed for potential control. Fibres from this muscle are sufficiently short (~ 1.5 mm) to allow fairly uniform changes in membrane potential when current is passed through an intracellular electrode in the middle of the fibre[9,10].

The activation of force after depolarization is subsequently followed by spontaneous relaxation (inactivation). Upon repolarization the ability to contract is restored in a relatively slow repriming process. During this period of mechanical refractoriness the activating intramembrane charges are supposed to be immobilized[7,11]. In a first series of experiments we measured the dependence of force repriming after a complete inactivation at -20 or zero mV membrane potential on potential and duration of the hyperpolarizing clamp pulse. From figure 1 it can be deduced that the speed of repriming increased drastically if the holding potential (HP) during repriming was shifted from -101 to -140 mV. At -101 mV almost no repriming occurred during the first 40 sec while it was complete after 20 sec at -140 mV. The results agree with those found in snake muscle fibres[12] and correspond to charge movement repriming in frog sartorius[11]. Figure 2a shows the dependence of force activation on depolarizing steps of a fibre, kept at a HP of -100 mV. The first signs of force development appeared between -50 and -40 mV and the maximum was reached near -30 mV, in good agreement with earlier K-contracture measurements. A 2nd fibre (b, c) was made mechanically refractory by depolarizing it to

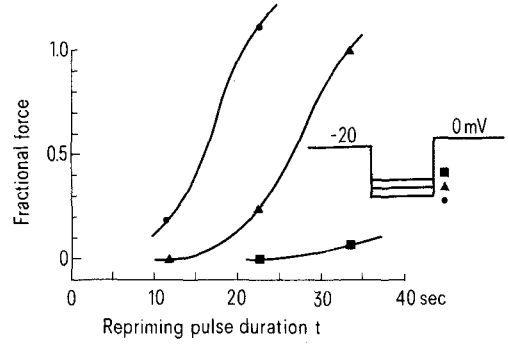

Fig. 1. The dependence of restoration (repriming) on holding potential (HP) and time. For repriming, the membrane was hyperpolarized from -20 mV to -101 (■), -118 (▲) and -140 (●) mV, successively. Force was in each case induced by a depolarizing step to zero mV (see inset). Ordinate: Fractional force, i.e. 1.0 is the mean of force development after depolarizing the fibre from -100 to zero mV at the beginning and the end of the experiment. Abscissa: Period of repriming in the hyperpolarized state. Between successive pulse protocols the membrane was clamped at -20 mV for 3–4 min. Ringer's solution contained (in mM): NaCl: 115; KCl: 2.5; Na_2HPO_4: 2.15; NaH_2PO_4: 0.85; $CaCl_2$: $1.8+2 \cdot 10^{-7}$ g/ml tetrodotoxin, 3.5 °C.