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Various Sodium Salts, Potassium Salts, a Calcium Salt and an Ammonium Salt Induced Ornithine Decarboxylase and Stimulated DNA Synthesis in Rat Stomach Mucosa

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Studies were made on the possible tumor-promoting activities of various salts of food additives in the glandular stomach mucosa of F344 male rats after their administration by gastric intubation. Up to 100-fold increases in ornithine decarboxylase (ODC) activity in the pyloric mucosa of the stomach with maxima after 8 h were observed after administration of sodium acetate at doses of 3.68–13.6 mmol/kg body weight, sodium L-ascorbate at doses of 8.55–17.1 mmol/kg body weight, Na_2CO_3 at doses of 4.73–14.2 mmol/kg body weight, sodium L-glutamate at doses of 12.8–17.1 mmol/kg body weight, sodium sorbate at doses of 8.92–17.1 mmol/kg body weight and $(NH_4)_2SO_4$ at doses of 7.56–20.1 mmol/kg body weight. Increases of up to 100-fold in ODC activity with maxima after 16 h were also observed after intubation of KCl at doses of 10.1–22.0 mmol/kg body weight, K_2SO_3 at doses of 2.84–8.45 mmol/kg body weight, $K_2S_2O_5$ at doses of 2.25–6.75 mmol/kg body weight and $CaCl_2$ at doses of 2.0–4.08 mmol/kg body weight. Sodium acetate at a dose of 11.0 mmol/kg body weight, KCl at a dose of 20.1 mmol/kg body weight, $K_2S_2O_5$ at a dose of 5.40 mmol/kg body weight and $CaCl_2$ at a dose of 3.4 mmol/kg body weight induced up to 10-fold increase in DNA synthesis in the pyloric mucosa of the stomach with maxima after 16–24 h. These results suggest that these salts of food additives may, like NaCl, have tumor-promoting activities in the pyloric mucosa of rat stomach.

Key words: Sodium salt — Potassium salt — Ornithine decarboxylase — DNA synthesis — Rat stomach mucosa

The incidence of stomach cancer is higher than that of any other type of cancer in Japan and some other countries.[1] As human stomach cancers take a long time to develop, not only tumor initiators but also tumor promoters are suggested to be important in stomach carcinogenesis. Thus, for prevention of stomach cancer, possible initiators and promoters of stomach tumors must be identified. Previously we reported a short-term *in vivo* method for evaluating the tumor-initiating activity of possible glandular stomach carcinogens with unscheduled DNA synthesis as a marker[2] and the tumor-promoting activity of possible glandular stomach tumor-promoters with ornithine decarboxylase (ODC) activity and DNA synthesis as markers.[3]

Takahashi *et al.* reported that NaCl and $K_2S_2O_5$ enhanced two-stage gastric carcinogenesis in rat stomach mucosa initiated by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG).[4,5] We reported that these two compounds[3,6] and other glandular stomach tumor promoters such as sodium taurocholate,[7] glyoxal[8] and formaldehyde[9] induced up to 100-fold increase of ODC activity and about 10-fold increase in DNA synthesis in the pyloric mucosa of rat stomach. We also reported that glandular stomach carcinogens such as MNNG, N-ethyl-N′-nitro-N-nitrosoguanidine (ENNG), N-propyl-N′-nitro-N-nitrosoguanidine (PNNG), 4-nitroquinoline 1-oxide (4NQO) and N-nitroso-N-methylurethane (NMUT) induced up to 100-fold increase of ODC activity and about 10-fold increase in DNA synthesis in the pyloric mucosa of rat stomach.[10] In this paper we deal with possible tumor-promoting activities of various salts of food additives other than NaCl and $K_2S_2O_5$ in the pyloric mucosa of rat stomach and show that various sodium salts, potassium salts, a calcium salt and an ammonium salt of food additives also induced ODC activity and DNA synthesis in the pyloric mucosa of rat stomach after their administration by gastric intubation. Sodium acetate is an additive for food preparation, sodium L-ascorbate (vitamin C) is added for nutrition, Na_2CO_3 is used as an alkali, sodium L-glutamate is a condiment, and sodium sorbate is used for preservation. $(NH_4)_2SO_4$ and $MgSO_4$ are additives for brewing, K_2SO_3 and $K_2S_2O_5$ are bleaching agents, and $CaCl_2$ is used for nutrition.

MATERIALS AND METHODS

Chemicals Sodium acetate trihydrate, Na_2CO_3, NaCl, sodium L-glutamate monohydrate, KCl, $K_2S_2O_5$, $CaCl_2 \cdot 2H_2O$, $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$ and saccharose (reagent grade) and K_2SO_3 (first grade) were purchased from Wako Pure Chemical Industries, Ltd., Osaka. Sodium

L-ascorbate and 3,5-diaminobenzoic acid dihydrochloride (DABA) (reagent grade) and sodium sorbate (first grade) were obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo. L-[1-^{14}C]Ornithine (55.9 mCi/mmol) and [methyl-^{3}H]thymidine ([^{3}H]dThd) (~80 Ci/mmol) were purchased from New England Nuclear, Boston, MA.

Animals Male Fischer rats (F344/DuCrj: Charles River Japan, Inc., Kanagawa), 7 to 8 weeks old, were kept individually in hanging wire-net cages and were given a limited amount of diet (4 g per rat) overnight to reduce their stomach contents. The following day they were given 0.5 to 1.0 ml of an aqueous solution of a test chemical by gastric intubation between 8 to 9 a.m. except in experiments for 16 h, in which rats were starved from the morning and were given a test chemical in the evening.

ODC activity Enzyme extracts were prepared from the pyloric mucosa of rat stomach after administration of a test chemical.[11] ODC activity was determined with L-[1-^{14}C]ornithine as a substrate, as described previously.[11] The protein content of the enzyme extract was determined by micro-assay as described by Heil and Zilling[12] with bovine serum albumin as a standard. Results are means of duplicate assays on pooled materials from four rats.

DNA synthesis DNA synthesis in the pyloric mucosa of the stomach was determined in *in vitro* organ culture in the presence of [^{3}H]dThd after administration of a test chemical *in vivo* as described previously.[2,11] The DNA fraction was extracted from the tissue and incorporation of [^{3}H]dThd into DNA was determined in a Beckman LS-355 liquid scintillation counter. The DNA content of the DNA fraction was determined with DABA as described previously[11] with calf thymus DNA as a standard.

RESULTS

Induction of ODC activity in the pyloric mucosa of rat stomach Figure 1 a–f shows the inductions of ODC activity in the pyloric mucosa of rat stomach after administration of (a) sodium L-ascorbate, (b) sodium L-glutamate, (c) KCl, (d) $K_2S_2O_5$, (e) $CaCl_2$, and (f) $(NH_4)_2SO_4$. ODC activity increased up to 100-fold with maxima 8 h after administrations of sodium and calcium salts and 16 h after administrations of potassium and ammonium salts, returning to the control level after 48 h.

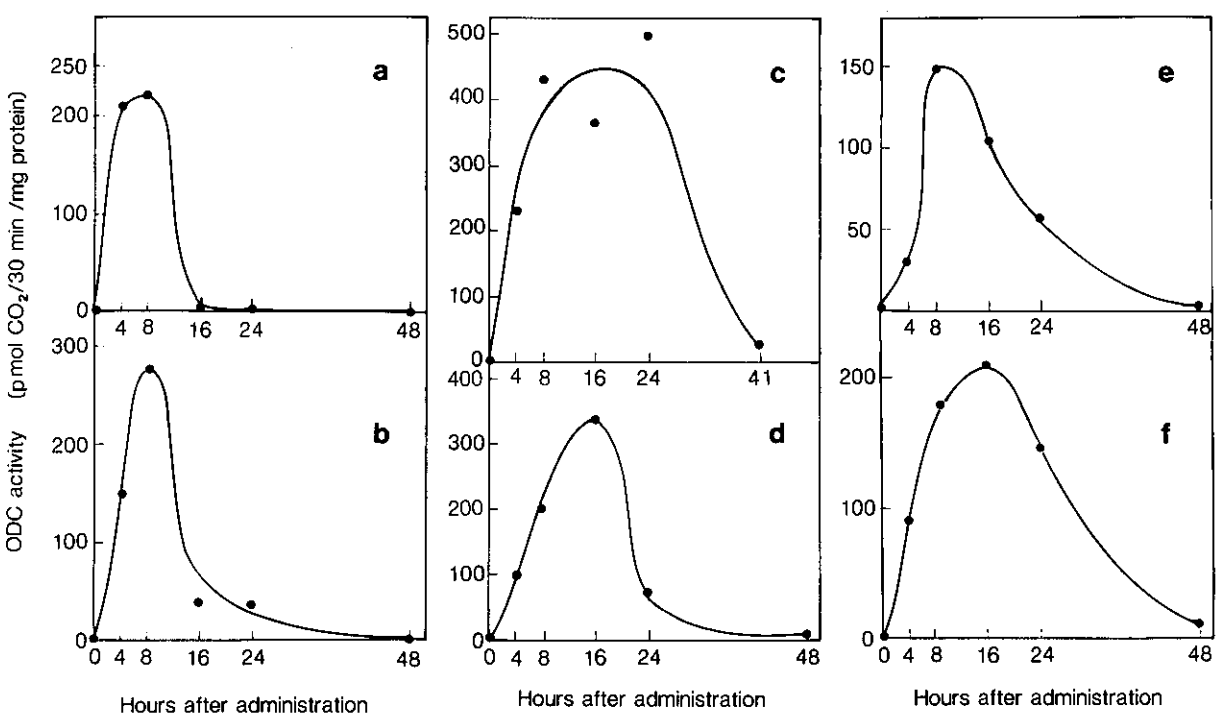

Fig. 1. Inductions of ODC activity in the pyloric mucosa of rat stomach after administration of a) sodium L-ascorbate (17.1 mmol/kg body weight), b) sodium L-glutamate (17.1 mmol/kg body weight), c) KCl (24.8 mmol/kg body weight), d) $K_2S_2O_5$ (6.75 mmol/kg body weight), e) $CaCl_2$ (4.08 mmol/kg body weight) and f) $(NH_4)_2SO_4$ (14.7 mmol/kg body weight). Results are means of duplicate assays on pooled materials from four rats.

Table I. Dose-dependent Inductions of ODC Activity in the Pyloric Mucosa of Rat Stomach by Salts

Chemical	Dose (g/kg body weight)	ODC activity[a] (pmol CO_2/30 min/mg protein)	Chemical	Dose (g/kg body weight)	ODC activity[a] (pmol CO_2/30 min/mg protein)
Na acetate·$3H_2O$	0	4		1.5	430
	0.5	32		1.64 (22.0 mmol)	634
	1.0	101	K_2SO_3	0	4
	1.5	390		0.45	25
	1.85 (13.6 mmol)	631		0.89	281
Na L-ascorbate	0	3		1.34 (8.45 mmol)	447
	0.85	8	$K_2S_2O_5$	0	4
	1.7	24		0.5	37
	2.5	38		0.8	75
	3.4 (17.1 mmol)	235		1.1	89
Na_2CO_3	0	1		1.4 (6.3 mmol)	94
	0.5	87	$CaCl_2·2H_2O$	0	0
	1.0	377		0.1	0
	1.5 (14.2 mmol)	478		0.3	13
NaCl[b]	0	5		0.5	176
	0.25	17		1.0	74
	0.5	45		1.5 (10.2 mmol)	16
	1.0	656	$(NH_4)_2SO_4$	0	5
	1.5 (25.7 mmol)	1238		0.5	16
Na L-glutamate·H_2O	0	3		1.0	67
	0.8	2		1.5	56
	1.6	8		2.66 (20.1 mmol)	367
	2.4	30	$MgSO_4·7H_2O$	0	0
	3.2 (17.1 mmol)	278		0.5	0
Na sorbate	0	1		1.0	0
	0.6	3		1.5	0
	1.2	20		2.0 (8.13 mmol)	1
	1.7	136	Saccharose	0	0
	2.3 (17.1 mmol)	417		1.1	0
KCl	0	0		2.3	0
	0.25	0		4.5 (13.2 mmol)	0
	0.75	34			

a) Results are means of duplicate assays on pooled materials from four rats. Effects were examined 8 h after administration of all chemicals except NaCl (examined after 6 h) and $K_2S_2O_5$ (examined after 16 h).
b) The results have been published as a figure.[3]

Table I shows the dose-dependent inductions of ODC activity in the pyloric mucosa of rat stomach by sodium acetate, sodium L-ascorbate, Na_2CO_3, sodium L-glutamate, sodium sorbate, KCl, K_2SO_3, $CaCl_2$ and $(NH_4)_2SO_4$, 8 h after their administration and by $K_2S_2O_5$, 16 h after its administration. The results with NaCl, given for reference in Table I, were shown as a figure in our previous paper.[3] We have also reported some of the results for $K_2S_2O_5$ in a previous paper.[6] As shown in Table I, ODC activity was not induced in the pyloric mucosa of rat stomach by $MgSO_4$ at doses up to 8.13 mmol/kg body weight. It was also not induced by saccharose at doses of up to 13.2 mmol/kg body weight, as also shown in Table I. The latter finding suggests that ODC activity was not induced in the pyloric mucosa of rat stomach by hypertonic solution but by electrolytes such as sodium, potassium, calcium and ammonium.

Increase of DNA synthesis in the pyloric mucosa of rat stomach Figure 2 a–d shows the increases of DNA synthesis in the pyloric mucosa of rat stomach after administrations of (a) KCl at a dose of 20.1 mmol/kg body weight, (b) $K_2S_2O_5$ at a dose of 5.40 mmol/kg body weight, (c) sodium acetate at a dose of 11.0 mmol/kg body weight, and (d) $CaCl_2$ at a dose of 3.40 mmol/kg body weight. DNA synthesis increased within 3–4 h after administration of these chemicals. The maximal in-

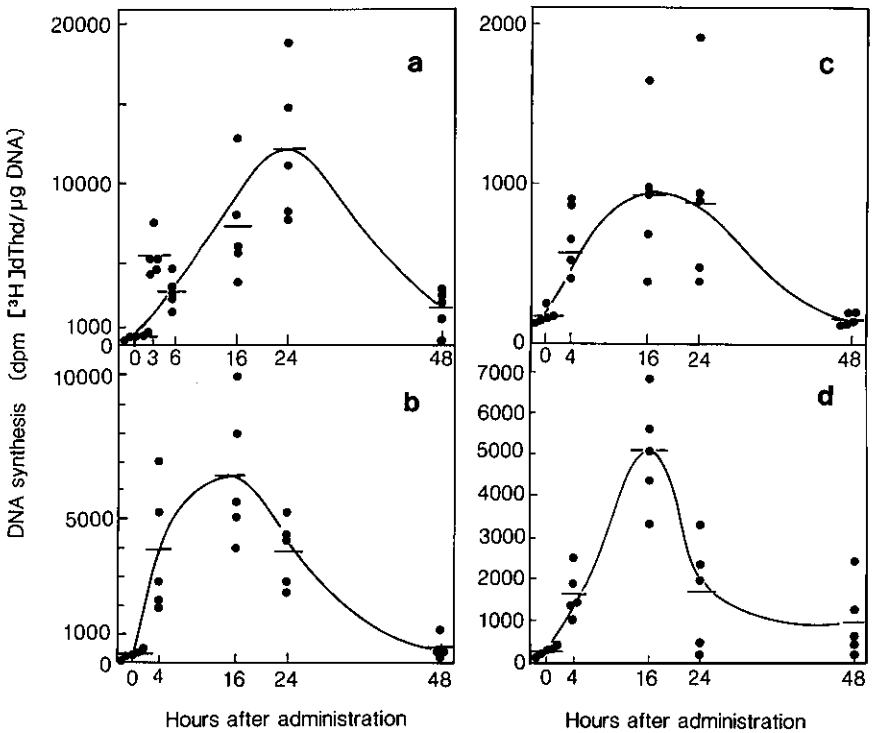

Fig. 2. Increases of DNA synthesis in the pyloric mucosa of rat stomach by salts. a) KCl (20.1 mmol/kg body weight), b) $K_2S_2O_5$ (5.4 mmol/kg body weight), c) sodium acetate (11.0 mmol/kg body weight), and d) $CaCl_2$ (3.4 mmol/kg body weight). Results are for five individual rats at each time. Values were significantly different from that at time 0 by Student's t test at 3 h ($P<0.001$), 6 h and 24 h ($P<0.01$) and 16 h ($P<0.02$) for KCl, at 4 h ($P<0.05$), 16 and 24 h ($P<0.01$) for $K_2S_2O_5$, at 4 h ($P<0.01$) and 16 h ($P<0.05$) for sodium acetate, and at 4 and 16 h ($P<0.01$) for $CaCl_2$.

Table II. Dose-dependent Increase in DNA Synthesis in the Pyloric Mucosa of Rat Stomach Induced by $K_2S_2O_5$

Dose (g/kg body weight)	DNA synthesis[a] (dpm [³H]dThd/μg DNA)	Significance (Student's t test)
0	152±38.5	
0.4	427±272	$P>0.05$
0.8	1169±821	$P>0.05$
1.2 (5.4 mmol)	2085±576	$P<0.01$

a) Means±SD of values in single assays on 5 rats at doses of 0, 0.4 and 0.8 g and on 4 rats at a dose of 1.2 g at 16 h after treatment.

creases, observed at 16–24 h, were 31-fold with KCl, 25-fold with $K_2S_2O_5$, 5.5-fold with sodium acetate, and 17-fold with $CaCl_2$ and the levels returned to the control level after 48 h. As replicative DNA synthesis is always observed in the proliferative zone of the pyloric mucosa of normal rat stomach, DNA synthesis at time 0 in Fig. 2 represents the control level of DNA synthesis in the pyloric mucosa of control rats.

Table II shows the dose-dependent increase of DNA synthesis in the pyloric mucosa of rat stomach 17 h after administration of $K_2S_2O_5$.

DISCUSSION

Previously we reported that 5 glandular stomach tumor promoters, NaCl,[3] $K_2S_2O_5$,[6] sodium taurocholate,[7] glyoxal[8] and formaldehyde[9] and 5 glandular stomach carcinogens, MNNG, ENNG, PNNG, 4NQO and NMUT[10] induced ODC activity and DNA synthesis in the pyloric mucosa of rat stomach. Glyoxal was tested as a glandular stomach tumor promoter[13] based on our previous suggestion that it might have tumor-promoting activity in the glandular stomach mucosa. We also reported that bile acids, such as sodium taurochenodeoxycholate, sodium taurodeoxycholate, sodium glycocholate, sodium glycochenodeoxycholate and sodium glycodeoxycholate,[7] dicarbonyl compounds such as methylglyoxal[11] and diacetyl,[8] and nitroso compounds such as 1-nitrosoindole-3-acetonitrile[6] and 3-diazo-N-nitroso-

bamethan[14] induced ODC activity and DNA synthesis in the pyloric mucosa of rat stomach and suggested their possible tumor-promoting activity in glandular stomach carcinogenesis.

The present study showed that various sodium salts, potassium salts, a calcium salt and an ammonium salt of food additives also induced ODC activity and DNA synthesis in the pyloric mucosa of rat stomach, like NaCl and $K_2S_2O_5$. These findings suggest that they may have tumor-promoting activities in glandular stomach carcinogenesis.

In 1984, the mean intake of NaCl in adult Japanese was 12.2 g (4.79 g as Na) per day, and their mean intakes of K and Ca were 3.67 g and 0.56 g/day, respectively. The risk of NaCl as a stomach tumor promoter in humans has been studied extensively, but the risks of other salts or electrolytes have not been reported. The present results suggest that the risks of other salts and electrolytes as stomach tumor promoters must also be examined. In the present study, the doses of all the salts tested except NaCl were much higher than the normal daily human intakes. Daily intake of food additives in Japanese was reported by the Ministry of Health and Welfare, Japan. Total intake of food additives of group A (non-natural additives), such as sorbate, was about 100 mg/day and that of group B (natural additives), such as vitamin C and glutamate, was about 10 g/day. Daily intake of sodium L-ascorbate and sodium L-glutamate may be g order/day. Daily intake of other food additives examined should be small. Therefore daily intake of sodium L-ascorbate and sodium L-glutamate by Japanese is less than $1/10^2$ of the dose that induced ODC in rat stomach mucosa. Daily intake of other food additives by Japanese is less than $1/10^3$ of the dose that induced ODC in rat stomach mucosa. However, attention must be paid to the total intake of various salts including NaCl in daily life. The effects of intakes of mixed salts including NaCl must also be examined.

Similar phenomena were observed in urinary bladder carcinogenesis. Various sodium salts, such as sodium ascorbate, sodium bicarbonate,[15] sodium saccharin,[16] sodium *o*-phenylphenate[17] and sodium erythorbate[18] were reported to promote urinary bladder carcinogenesis. Increase of the sodium ion concentration and pH of the urine are apparently important factors for urinary bladder carcinogenesis.

In the present study rats were given a limited amount of diet overnight to reduce their dietary stomach contents in order to observe the effects of chemicals clearly. When rats were given a diet supplemented with 10% NaCl for 8 months, up to 10-fold increase in ODC activity in the pyloric mucosa of the stomach was observed (C. Furihata and M. Takahashi, unpublished).

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