



# Na<sup>+</sup>/Ca<sup>2+</sup> exchanger contributes to stool transport in mice with experimental diarrhea

Kazuhiro NISHIYAMA<sup>1) #</sup>, Kohta TANIOKA<sup>1) #</sup>, Yasu-Taka AZUMA<sup>1) \*</sup>,  
Satomi HAYASHI<sup>1)</sup>, Yasuyuki FUJIMOTO<sup>1)</sup>, Natsuho YOSHIDA<sup>1)</sup>, Satomi KITA<sup>2)</sup>,  
Sho SUZUKI<sup>1)</sup>, Hidemitsu NAKAJIMA<sup>1)</sup>, Takahiro IWAMOTO<sup>2)</sup> and  
Tadayoshi TAKEUCHI<sup>1)</sup>

<sup>1)</sup>Laboratory of Veterinary Pharmacology, Division of Veterinary Science, Osaka Prefecture University Graduate School of Life and Environmental Science, 1-58 Rinku-ohraikita, Izumisano, Osaka 598-8531, Japan

<sup>2)</sup>Department of Pharmacology, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

**ABSTRACT.** The Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) is a bidirectional transporter that is controlled by membrane potential and transmembrane gradients of Na<sup>+</sup> and Ca<sup>2+</sup>. To reveal the functional role of NCX on gastrointestinal motility, we have previously used NCX1 and NCX2 heterozygote knockout mice (HET). We found that NCX1 and NCX2 play important roles in the motility of the gastric fundus, ileum and distal colon. Therefore, we believed that NCX1 and NCX2 play an important role in transport of intestinal contents. Here, we investigated the role of NCX in a mouse model of drug-induced diarrhea. The fecal consistencies in NCX1 HET and NCX2 HET were assessed using a diarrhea induced by magnesium sulfate, 5-hydroxytryptamine (5-HT) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). NCX2 HET, but not NCX1 HET, exacerbated magnesium sulfate-induced diarrhea by increasing watery fecals. Likewise, 5-HT-induced diarrheas were exacerbated in NCX2 HET, but not NCX1 HET. However, NCX1 HET and NCX2 HET demonstrated PGE<sub>2</sub> induced diarrhea similar to those of wild-type mice (WT). As well as the result of the distal colon shown previously, in the proximal and transverse colons of WT, the myenteric plexus layers and the longitudinal and circular muscle layers were strongly immunoreactive to NCX1 and NCX2. In this study, we demonstrate that NCX2 has important roles in development of diarrhea.

**KEY WORDS:** diarrhea, 5-hydroxytryptamine, magnesium sulphate, myenteric neurons, na<sup>+</sup>/Ca<sup>2+</sup> exchanger

*J. Vet. Med. Sci.*

79(2): 403–411, 2017

doi: 10.1292/jvms.16-0475

Received: 10 September 2016

Accepted: 25 November 2016

Published online in J-STAGE:

8 December 2016

Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) is an exchanger of Na<sup>+</sup> and Ca<sup>2+</sup> across the membrane by electrogenical membrane potential and transmembrane gradients. The functional roles by which NCX influences gastrointestinal motility including contraction and relaxation are incompletely understood and vary by tissue, although its role in cardiac muscle and brain neurons is well understood. Our group is showing to reveal the functional role of NCX on gastrointestinal motility, because Ca<sup>2+</sup> homeostasis is central to the regulation of gastrointestinal smooth muscle functions [1, 4, 7, 12–14]. To specifically assess the physiological role of NCX, we used NCX1 heterozygote knockout mice (NCX1 HET) and NCX2 HET. To improve the understanding of NCX action on gastrointestinal motility, we examined to characterize the motilities in gastrointestinal segments from the gastric fundus, ileum and distal colon in NCX1 HET and NCX2 HET using an organ tissue bath system. In these previous articles, we found that NCX1 and NCX2 play important roles in the motility in the gastric fundus [4], ileum [14] and distal colon [1, 13], although NCX1 HET and NCX2 HET appeared outwardly healthy and displayed normal growth established by body weight changes, amount of drinking, food intake, fecal weights and fecal numbers. Therefore, we believed that NCX1 and NCX2 play an important role in transport of intestinal contents. However, the pathological role of NCX on the constipation and diarrhea is little known. We considered that analysis of NCX in the colon might lead to the development of new treatments and a prevention method for constipation and diarrhea. In this study, we investigated the susceptibility of NCX HET to the development of three models of drug-induced diarrhea. Magnesium sulphate induces diarrhea by causing an increase in the osmotic pressure in the intestinal tract

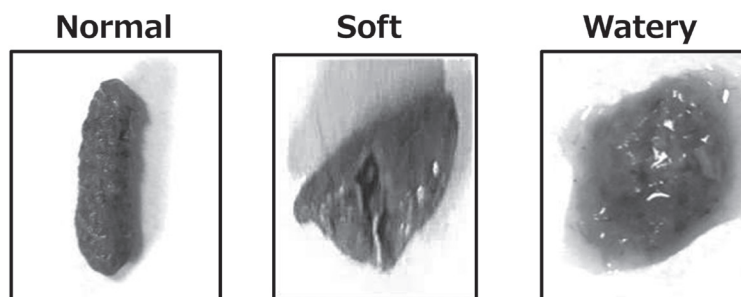
\*Correspondence to: Azuma, Y.T., Laboratory of Veterinary Pharmacology, Division of Veterinary Science, Osaka Prefecture University Graduate School of Life and Environmental Science, 1-58 Rinku-ohraikita, Izumisano, Osaka 598-8531, Japan. e-mail: azuma@vet.osakafu-u.ac.jp

#Equally contribution.

©2017 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/4.0/>>.



**Fig. 1.** The fecals were graded into three consistency levels as follows: normal, soft and watery. A three-point rating system was used to characterize diarrhea: normal, normal brown formed stool; soft, soft brown stool & soft-mucous brown yellow stool; watery, muddy-mucous yellow stool & liquid-mucous yellow stool.

[10]. 5-Hydroxytryptamin (5-HT) induces diarrhea by stimulating the cholinergic and tachykininergic excitatory pathways [6, 17]. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) induces diarrhea by stimulating the accumulation of fluid in the colon [15].

## MATERIALS AND METHODS

### Drugs

Atropine, N-nitro-L-arginine (L-NNA), magnesium sulfate and PGE<sub>2</sub> were purchased from Wako Pure Chemical (Osaka, Japan). 5-HT was purchased from Tokyo chemical industry (Tokyo, Japan). An Alexa Fluor 488-labeled goat anti-rabbit IgG and an Alexa Fluor 568-labeled goat anti-mouse IgG were purchased from Molecular Probes Inc. (Eugene, OR, U.S.A.). Rabbit polyclonal antibodies against NCX1 and NCX2 were produced as described previously [9]. A mouse polyclonal antibody against PGP9.5 was purchased from UltraClone Limited (Wellow, U.K.).

### Animals

NCX1 HET and NCX2 HET were produced as reported previously [8, 18]. These mice (9–12 weeks old) on the C57BL/6 background were comparable in all analyses to age-matched WT. All procedures used in this study were performed according to the institutional policies of the Osaka Prefecture University Animal Care and Use Committee.

### Drugs-induced diarrhea

We used two mice of the same genotype in one cage. Data from one cage count one number of an experiment. After the administration of the diarrheogenic drugs, magnesium sulfate (2 g/kg, p.o.), 5-HT (1 mg/kg, i.p.) and PGE<sub>2</sub> (0.3 mg/kg, i.p.), the fecals consistency was noted [6, 10, 15, 16]. The fecals were graded into three consistency levels as follows: normal, soft and watery (Fig. 1). In particular, a three-point rating system was used to characterize diarrhea: normal, normal brown formed stool; soft, soft brown stool & soft-mucous brown yellow stool; and watery, muddy-mucous yellow stool & liquid-mucous yellow stool.

### Expressions of NCX1 and NCX2

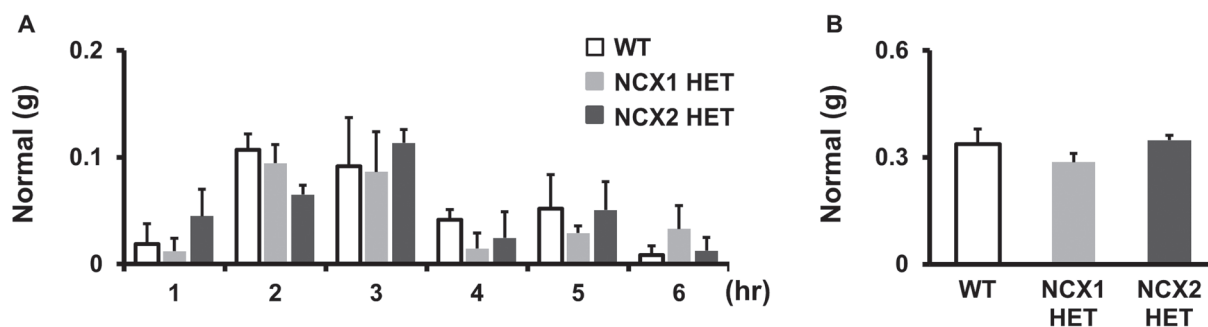
We analyzed expressions of NCX1 and NCX2 using quantitative real-time PCR as described previously [3] with some modifications [1]. Briefly, total RNA was extracted from the proximal and transverse colons. The primers used for amplification of NCX1 and NCX2 were as follows: 5'-CCTTGTGCATCTTAGCAATG-3' and 5'-TCTCACTCATCTCCACCAGA-3', and 5'-ATGGCTCCCTTGGCTTTGATG-3' and 5'-CAGCGGTAGGAACCTTGGC-3', respectively. Amplification of hypoxanthine phosphoribosyltransferase (HPRT) mRNA was used for each experimental sample as an endogenous control to account for differences in the amount and quality of total RNA added to each reaction.

### Immunofluorescent staining

Immunofluorescent staining for frozen sections was performed as described previously [5] with some modifications [11]. Briefly, mice were fixed by transcardiac perfusion with 4% paraformaldehyde, and the proximal and transverse colons were removed. Frozen sections (5 μm thick) were cut and prepared for immunofluorescent staining. The immunoreactivity of NCX1 and NCX2 was detected using an Alexa Fluor 488-labeled goat anti-rabbit IgG antibody. To detect neurons, the immunoreactivity of PGP9.5 was detected using an Alexa Fluor 568-labeled goat anti-mouse IgG antibody. Confocal images were obtained under a laser-scanning microscope (C1si; Nikon Corporation, Tokyo, Japan).

### Motility in proximal and transverse colons

*Ex vivo* motility was analyzed using previously described methods [7]. Briefly, the proximal and transverse colons were removed from mice (10–15 weeks old). Whole-wall strips were prepared in the orientation of the longitudinal muscle layer. The strips were exposed to electrical field stimulation (EFS) with 100 pulses for 10 sec. Atropine (1 μM) or L-NNA (30 μM) was added at least



**Fig. 2.** Fecal weights in saline administrated mice. Fecal samples were collected at various times for up to 6 hr beginning immediately after the administration of saline. Weight of normal fecal was measured (A). Total weight of each fecal is shown (B). Data include WT (n=3), NCX1 HET (n=3) and NCX2 HET (n=3).

10 min prior to EFS. Contraction or relaxation was analyzed by measuring the extent of the maximal contraction in response to 60 mM KCl or the extent of the maximal relaxations in response to Ca<sup>2+</sup>-free EGTA solution.

### Statistical analysis

The results are expressed as the means  $\pm$  S.E. Statistical significance was determined using one-way ANOVA for non-repeated measures to detect differences among WT, NCX1 HET and NCX2 HET. The differences between groups were determined using the Dunnett test. A P value of less than 0.05 was considered significant.

## RESULTS

### Magnesium sulfate-induced diarrhea

We confirmed that WT, NCX1 HET and NCX2 HET administered with saline had only normal fecal but not soft and watery fecals. Normal fecal weights after saline administration were similar among WT, NCX1 HET and NCX2 HET (Fig. 2).

In WT, magnesium sulfate induced soft and watery fecals as diarrhea (Fig. 3). Normal fecal weight was decreased in NCX1 HET at 6 hr and in NCX2 HET at 4, 5 and 6 hr after magnesium sulfate administration (Fig. 3A). NCX1 HET exhibited soft fecal weight similar to those of WT (Fig. 3B). Like normal fecal, soft fecal weight was decreased in NCX2 HET at 5 hr after magnesium sulfate administration (Fig. 3B). However, total weights of normal and soft fecals during 6 hr in NCX1 HET and NCX2 HET after magnesium sulfate administration were similar to those observed in WT (Fig. 3D and 3E). In contrast, watery fecal weight was increased in NCX1 HET at 6 hr after magnesium sulfate administration (Fig. 3C). Watery fecal weight was increased in NCX2 HET at 4 hr after magnesium sulfate administration (Fig. 3C). Total weight of watery fecal during 6 hr was increased in NCX2 HET, but not NCX1 HET, compared to WT (Fig. 3F).

There is possibility that water drinking is increased in NCX2 HET after the administration of magnesium sulfate, then resulting in the increase of watery fecal. We monitored the amount of drinking of water and taking of food for the assessment of fecal state during 8 hr after the administration of magnesium sulfate. As shown in Fig. 4, there are no changes in the amounts of drinking of water and taking of food in NCX1 HET and NCX2 HET.

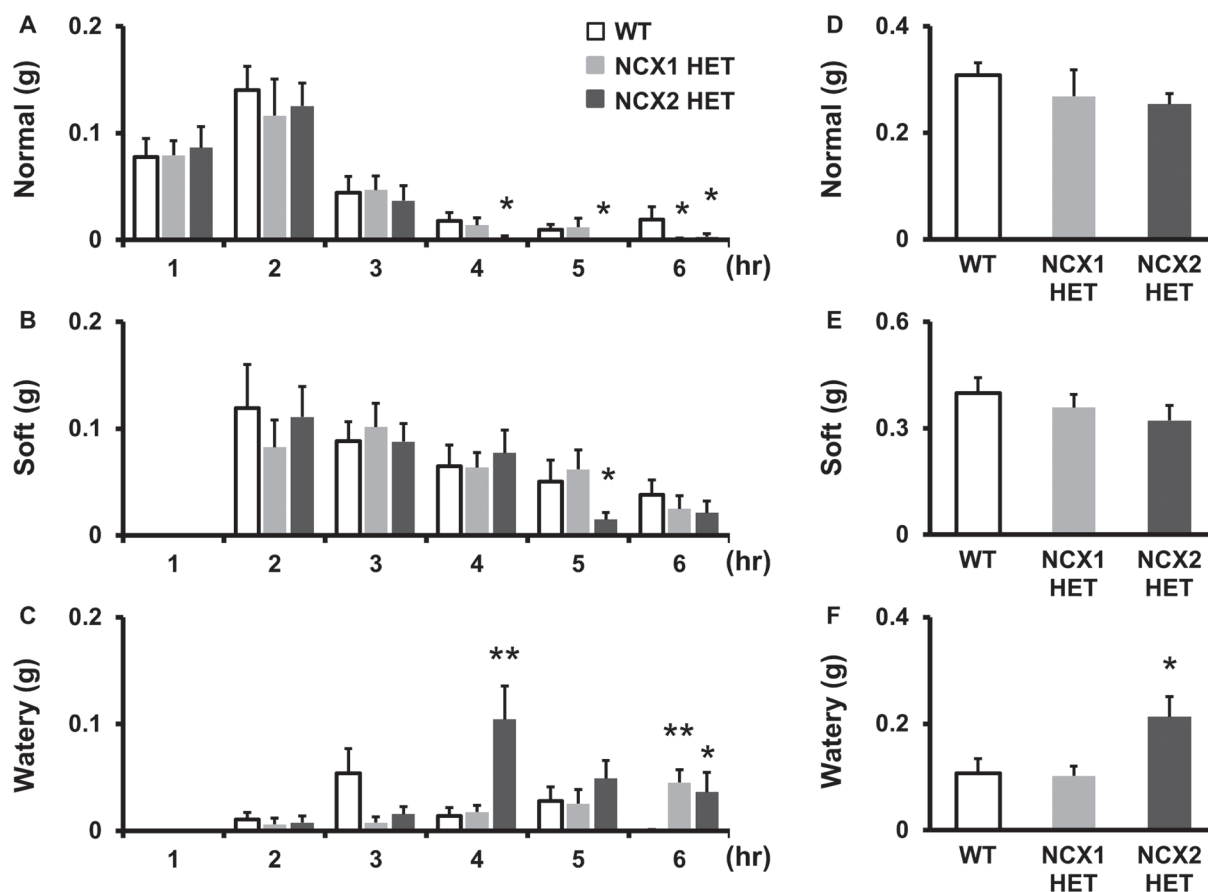
The movements of the contents in the gastrointestinal tract rely on the coordinated contractions and relaxations of the smooth muscles. ACh is most important transmitter on the motility in the gastrointestinal tract. We determined whether atropine affects magnesium sulfate-induced diarrhea. In WT, atropine (3 mg/kg) administered intraperitoneally 15 min before the administration of magnesium sulfate markedly inhibited magnesium sulfate-induced diarrhea (Fig. 5). Soft fecal weight was increased in NCX1 HET at 4 and 5 hr. NCX1 HET showed fecal weights of watery in a manner similar to WT. Watery fecal weight was increased in NCX2 HET at 4 and 8 hr in accordance with decrease of soft fecal weight at 8 hr. (Fig. 5). Indeed, atropine did not completely inhibit diarrhea in NCX1 HET and NCX2 HET. The peak of watery fecal weight seemed to be delayed in NCX2 HET compared to the experiment without atropine.

### 5-HT-induced diarrhea

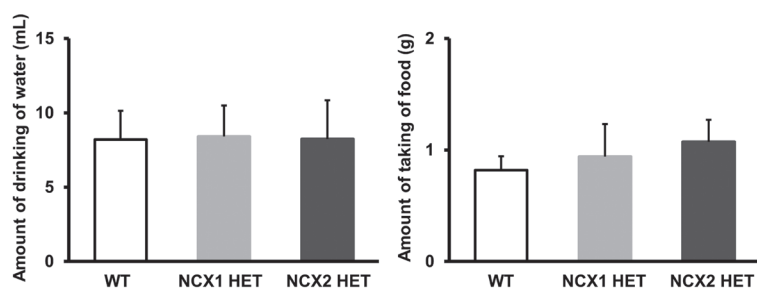
To further evaluate the role of NCX on stool transport in the colon, we use a 5-HT-induced diarrhea model that is different mechanism from magnesium sulfate. In WT, 5-HT induced soft and watery fecals as diarrhea (Fig. 6). Weights of normal and soft fecals in NCX1 HET and NCX2 HET were similar to those observed in WT (Fig. 6A and 6B). Weight of watery fecal was increased in NCX2 HET, but not NCX1 HET, compared to WT (Fig. 6C).

### PGE<sub>2</sub>-induced diarrhea

As mentioned above, magnesium sulfate- and 5-HT-induced diarrheas are correlated with enteric nervous system including myenteric neurons and interneurons [6, 10, 17]. To further demonstrate the possible mechanism of NCX on diarrhea models, we



**Fig. 3.** Magnesium sulfate-induced diarrhea. Fecal samples were collected at various times for up to 6 hr beginning immediately after the administration of magnesium sulfate. Weights of normal fecal (A), soft fecal (B) and watery fecal (C) were measured. Total weight of each fecal is shown (D,E,F). Data include WT (n=12), NCX1 HET (n=9) and NCX2 HET (n=9). \* $P < 0.05$  and \*\* $P < 0.01$  compared with WT.



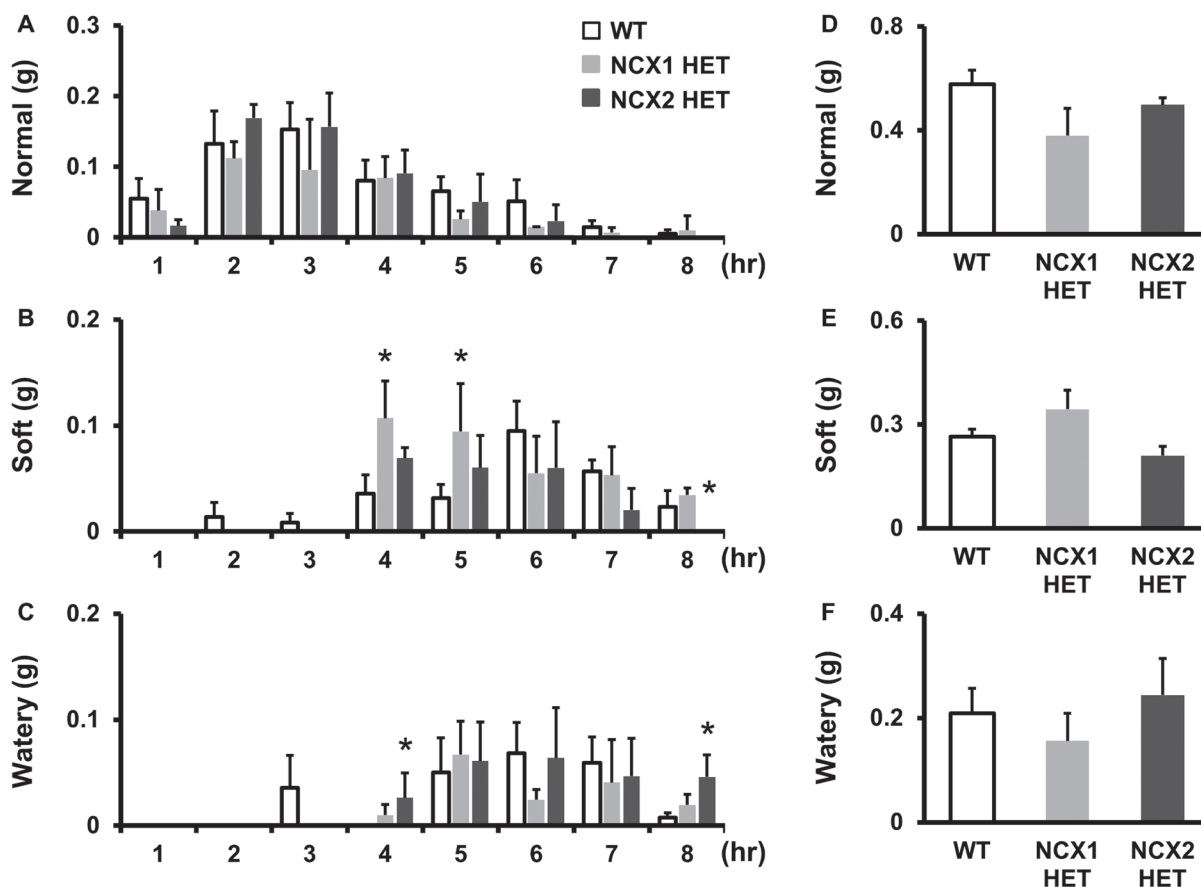
**Fig. 4.** Amounts of drinking of water and taking of food during 8 hr after the administration of magnesium sulfate. Data include WT (n=5), NCX1 HET (n=5) and NCX2 HET (n=4).

selected a PGE<sub>2</sub>-induced diarrhea model. Enteric nervous system is not involved in a PGE<sub>2</sub>-induced diarrhea model [15]. Therefore, we expected that NCX1 HET and NCX2 HET are similar to fecal weights observed in WT.

In WT, PGE<sub>2</sub> induced soft and watery fecals as diarrhea (Fig. 7). Weights of normal and soft fecals in NCX1 HET and NCX2 HET were similar to those observed in WT (Fig. 7A and 7B). Unlike magnesium sulfate and 5-HT, NCX1 HET and NCX2 HET demonstrated watery fecal weight similar to those of WT (Fig. 7C).

#### Localization of NCX1 and NCX2

Proximal and transverse colons are important to absorb most of the water present in the feces. In contrast, distal colon is important to store feces that will be emptied into the rectum. In a previous article [4], we showed the expression and localization



**Fig. 5.** Magnesium sulfate-induced diarrhea in the presence of atropine. Fecal samples were collected at various times for up to 8 hr beginning immediately after the administration of magnesium sulfate. Atropine (3 mg/kg) administered intraperitoneally 15 min before the administration of magnesium sulfate. Weights of normal fecal (A), soft fecal (B) and watery fecal (C) were measured. Total weight of each fecal is shown (D,E,F). Data include WT (n=6), NCX1 HET (n=3) and NCX2 HET (n=3). \* $P < 0.05$  compared with WT.

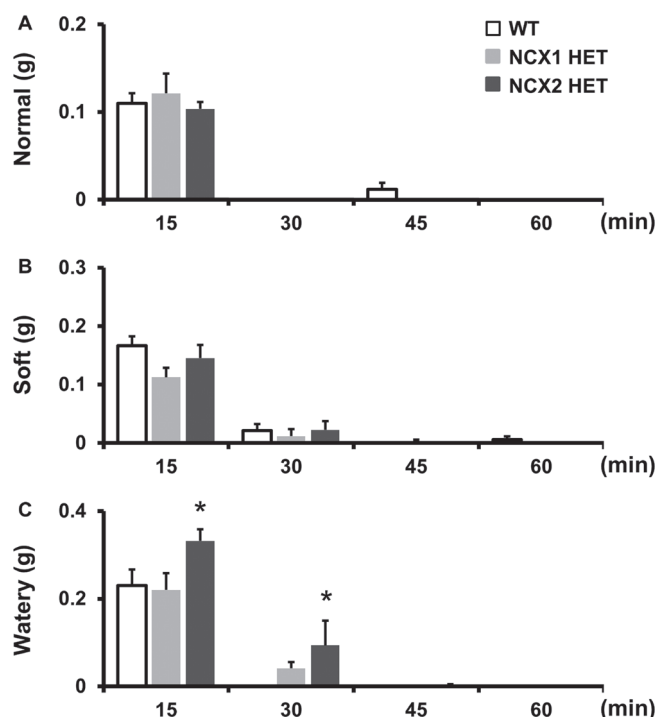
of NCX1 and NCX2 in the distal colon of WT using immunofluorescent staining. Therefore, we next investigated the expression and localization of NCX1 and NCX2 in the proximal and transverse colons of WT. As shown in Fig. 8A, expression of NCX1 and NCX2 was observed within the myenteric plexus layers. Strong immunoreactivity of NCX1 and NCX2 was also observed in the longitudinal and circular muscle layers. To estimate the histological change of NCX1- and NCX2-HET tissue preparations in the proximal and transverse colons, we dyed myenteric neurons with the PGP9.5 antibody. As shown in Fig. 8B, immunoreactivity of PGP9.5 was observed within the myenteric plexus layers in the proximal and transverse colons of NCX1 HET and NCX2 HET. There are no marked histological changes in the proximal and transverse colons between WT and NCXs HET.

#### Decreased expression of NCX1 and NCX2

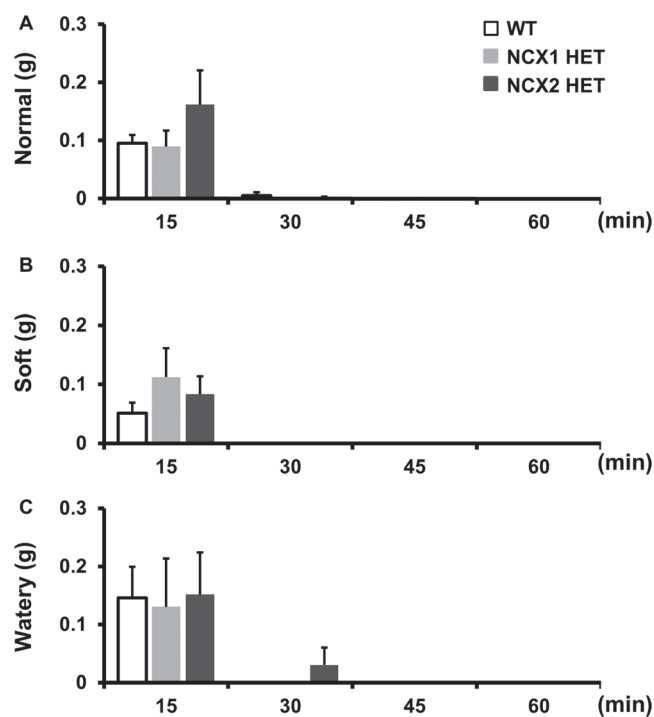
Like immunofluorescent staining, we showed that the expression level of NCX1 was significantly lower in the distal colon of NCX1 HET, whereas the distal colon of NCX2 HET expressed significantly lower levels of NCX2 [4]. We furthermore investigated the expressions of NCX1 and NCX2 in the proximal and transverse colons of NCX1 HET and NCX2 HET using quantitative real-time PCR. As shown in Fig. 9, the mRNA expression level of NCX1 was significantly lower in the proximal and transverse colons of NCX1 HET, whereas NCX2 HET expressed significantly lower levels of NCX2 mRNA in the proximal and transverse colons.

#### Altered motility

We investigated the response to EFS in the longitudinal muscles obtained from the proximal and transverse colons of WT, NCX1 HET and NCX2 HET. In WT, EFS induced a contraction in both proximal and transverse colons (Fig. 10 left panels). In the proximal colon, the magnitudes of the contraction were smaller in NCX1 HET and NCX2 HET than in WT. During the administration of L-NNA, an inhibitor of NO synthase, NCX1 HET and NCX2 HET showed a contraction in a manner similar to WT (Fig. 10, right upper panel). In contrast, the magnitudes of the contraction in the transverse colon were not significantly smaller in NCX1 HET than in WT. Importantly, NCX2 HET showed relaxation, but not contraction. In the presence of atropine, relaxations were greater in NCX1 HET and NCX2 HET than in WT (Fig. 10, right lower panel).



**Fig. 6.** 5-HT-induced diarrhea. Fecal samples were collected at various times for up to 60 min beginning immediately after the administration of 5-HT. Weights of normal fecal (A), soft fecal (B) and watery fecal (C) were measured. Data include WT (n=6), NCX1 HET (n=6) and NCX2 HET (n=6). \**P*<0.05 compared with WT.



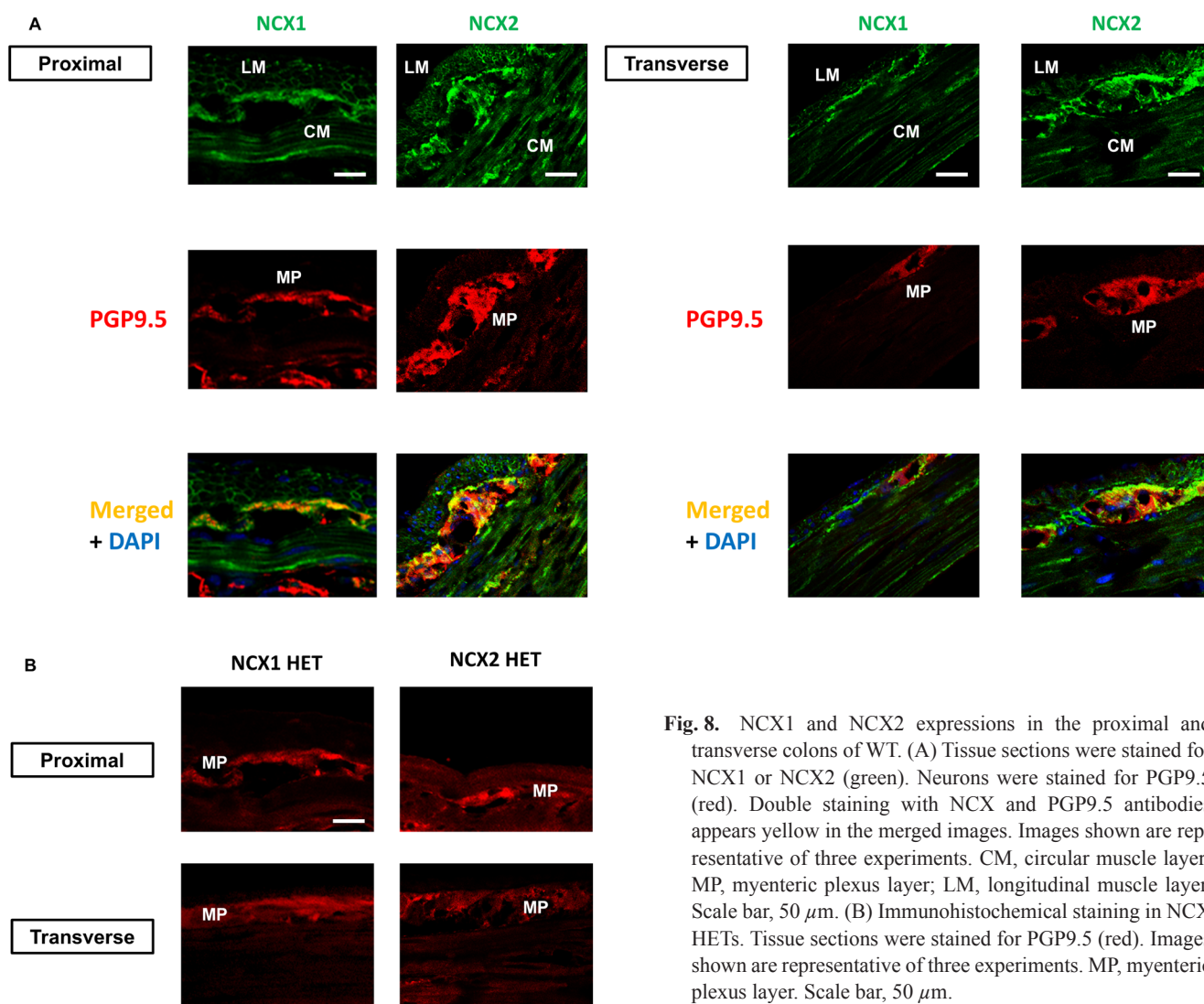
**Fig. 7.** PGE<sub>2</sub>-induced diarrhea. Fecal samples were collected at various times for up to 60 min beginning immediately after the administration of PGE<sub>2</sub>. Weights of normal fecal (A), soft fecal (B) and watery fecal (C) were measured. Data include WT (n=6), NCX1 HET (n=4) and NCX2 HET (n=4).

## DISCUSSION

The first aim of this study was to investigate the role of NCX in stool transport under the diarrhea. We showed that magnesium sulfate-induced diarrhea was exacerbated in NCX2 HET by decreasing normal and soft fecals and increasing watery fecal. Like magnesium sulfate-induced diarrhea, 5-HT-induced was also exacerbated in NCX2 HET. These results suggest that NCX2, but not NCX1, involves in some diarrhea. Total weight of watery fecal during 6 hr after magnesium sulfate administration was not increased in NCX1 HET compared to WT, although watery fecal weight was increased in NCX1 HET at 6 hr after magnesium sulfate administration. Unlike NCX2, weight of watery fecal after 5-HT administration was not increased in NCX1 HET compared to WT. These results suggest that NCX1 and NCX2 have different responses in stool transport under the diarrhea.

We think the possibility for severe diarrhea as observed in NCX2 HET. One possibility is that the altered motilities in stomach, small intestine and colon of NCX2 HET link severe diarrhea. In the presence of atropine, NCX2 HET shows delayed peak of diarrhea induced by magnesium sulfate compared to WT. In general, atropine inhibits the gastrointestinal motility. Therefore, it seems that severe diarrhea as observed in NCX2 HET is not potentially correlated with the motility mediated with cholinergic pathways. In the proximal colon, decreased contraction, as observed in NCXs HET, is likely due to an increased release of NO in myenteric neurons during EFS. We found that EFS-induced contraction in the presence of L-NNA was not small in the proximal colon of NCX1 HET and NCX2 HET, which suggests that the decreased contraction in NCX HETs is associated with NO. Similarly, we found that EFS-induced relaxation in the presence of atropine was greater in the transverse colon of NCX1 HET and NCX2 HET than in WT, which also suggests that the relaxation in NCX2 HET is associated with NO. NCX1 HET showed no significant changes in the transverse colon, although NCX1 HET showed similar responses to NCX2 HET in the proximal colon. It seems the motility in the transverse colon is correlated with severe diarrhea rather than the motility in the proximal colon. These results suggest that severe diarrhea as observed in NCX2 HET is correlated with the motility altering NO-mediated component.

In addition to magnesium sulfate, the difference between 5-HT and PGE<sub>2</sub> might provide us with a good suggestion on the mechanism of severe diarrhea in NCX2 HET. 5-HT induced diarrhea by stimulating the cholinergic and tachykinergic excitatory pathways via 5-HT<sub>3</sub> receptors within the myenteric plexus neurons [6, 17]. In contrast, PGE<sub>2</sub> induced diarrhea by the direct effect on smooth muscles as well as by stimulating the accumulation of fluid [15, 19]. Therefore, NCX2 expressed in the myenteric neurons is correlated with increased watery fecal in the 5-HT-induced diarrhea. In contrast, it is unlikely NCX2 expressed in the smooth muscles contributes to PGE<sub>2</sub>-induced diarrhea. From this point of view, the role of NCX2 expressed in the myenteric neurons may differ from that in smooth muscles.



**Fig. 8.** NCX1 and NCX2 expressions in the proximal and transverse colons of WT. (A) Tissue sections were stained for NCX1 or NCX2 (green). Neurons were stained for PGP9.5 (red). Double staining with NCX and PGP9.5 antibodies appears yellow in the merged images. Images shown are representative of three experiments. CM, circular muscle layer; MP, myenteric plexus layer; LM, longitudinal muscle layer. Scale bar, 50  $\mu$ m. (B) Immunohistochemical staining in NCX HETs. Tissue sections were stained for PGP9.5 (red). Images shown are representative of three experiments. MP, myenteric plexus layer. Scale bar, 50  $\mu$ m.

In this study and previous articles, we showed that the expression of NCX2 is all decreased in gastric fundus, ileum, proximal colon, transverse colon and distal colon of NCX2 HET. However, we did not check the expression of NCX2 in the myenteric neurons or smooth muscles of NCX2 HET. Indeed, we do not conclude which region and which types of cell can contribute to severe diarrhea in NCX2 HET. However, we are positive that colon is more important than stomach and small intestine because of the action points of magnesium sulfate and 5-HT [16]. Actually, immunostaining for NCX2 protein in the proximal and transverse colons showed that NCX2 was expressed in the smooth muscles and myenteric neurons in these colons. These findings suggest that NCX2 in the proximal and transverse colons as well as the distal colon may play an important role in stool transport under the diarrhea.

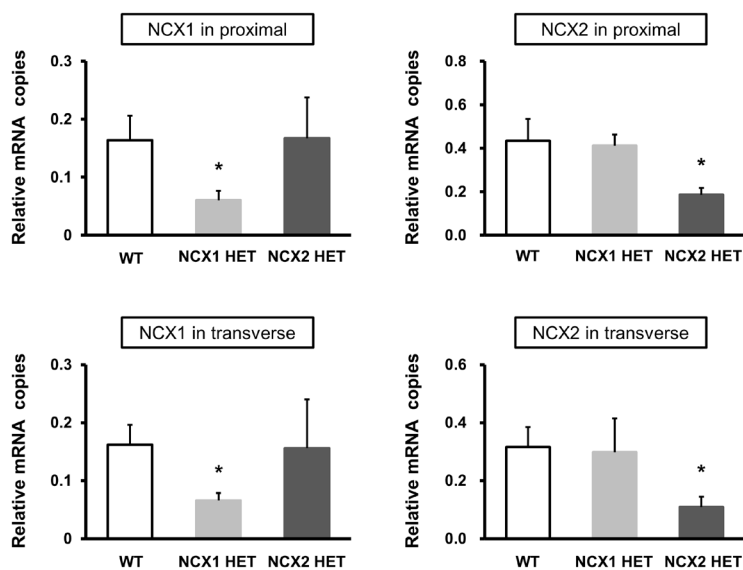
In conclusion, we demonstrated for first time using mouse diarrhea models that NCX2 has important roles in development of diarrhea. Diarrhea as well as constipation results in an uncomfortable feeling impressing the quality of life. Functional bowel disorder is defined as a disorder with symptoms attributable to the middle or lower gastrointestinal tract, including functional constipation, functional diarrhea and irritable bowel syndrome. The deficiency of NCX2 may cause functional diarrhea, followed by much discomfort. Therefore, the roles of NCX in gastrointestinal disorders may be an interesting topic of future research.

#### CONFLICT OF INTEREST

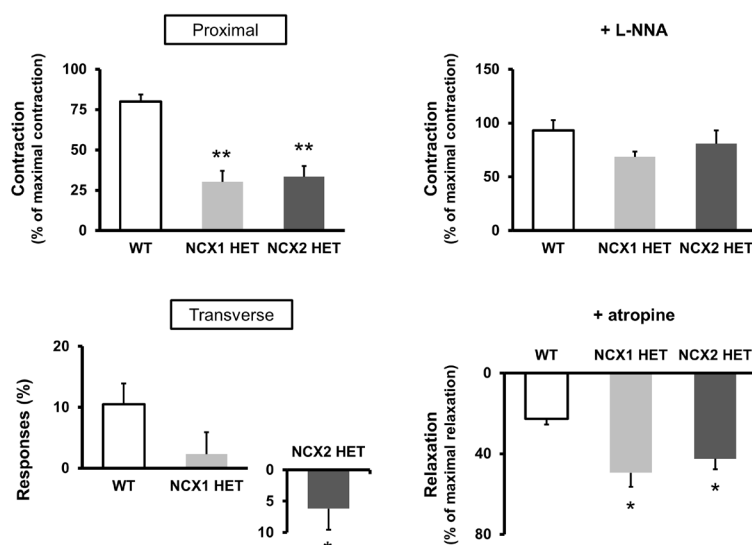
The authors declare that they have no conflicts of interest to declare.

#### REFERENCES

1. Azuma, Y. T., Nishiyama, K., Kita, S., Komuro, I., Nakajima, H., Iwamoto, T. and Takeuchi, T. 2012. Na<sup>(+)</sup>/Ca<sup>(2+)</sup> exchanger 2-heterozygote knockout mice display decreased acetylcholine release and altered colonic motility *in vivo*. *Neurogastroenterol. Motil.* **24**: e600–e610. [Medline] [CrossRef]



**Fig. 9.** NCX1 and NCX2 expressions in the proximal and transverse colons. The expression levels of NCX1 and NCX2 in WT (n=6), NCX1 HET (n=6) and NCX2 HET (n=6) were examined by quantitative real-time PCR. The mRNA level of NCX1 and NCX2 is shown as a fold increase relative to the level of HPRT mRNA. \* $P < 0.05$  compared with WT.



**Fig. 10.** EFS-induced responses in NCX HETs. EFS-induced responses in longitudinal muscle strips isolated from the proximal and transverse colons in WT (n=6–9), NCX1 HET (n=4–6) and NCX2 HET (n=5–10). Quantitative data on the contraction or relaxation during EFS are shown. Contractions were expressed as percentages of 60 mM KCl-induced contraction, and relaxations are expressed as percentages of  $\text{Ca}^{2+}$ -free EGTA-induced relaxation. \* $P < 0.05$  and \*\* $P < 0.01$  compared with WT.

- Azuma, Y. T., Nishiyama, K., Matsuo, Y., Kuwamura, M., Morioka, A., Nakajima, H. and Takeuchi, T. 2010. PPAR $\alpha$  contributes to colonic protection in mice with DSS-induced colitis. *Int. Immunopharmacol.* **10**: 1261–1267. [Medline] [CrossRef]
- Azuma, Y. T., Hagi, K., Shintani, N., Kuwamura, M., Nakajima, H., Hashimoto, H., Baba, A. and Takeuchi, T. 2008. PACAP provides colonic protection against dextran sodium sulfate induced colitis. *J. Cell. Physiol.* **216**: 111–119. [Medline] [CrossRef]
- Azuma, Y. T., Hayashi, S., Nishiyama, K., Kita, S., Mukai, K., Nakajima, H., Iwamoto, T. and Takeuchi, T. 2016.  $\text{Na}^{+}/\text{Ca}^{2+}$  exchanger-heterozygote knockout mice display increased relaxation in gastric fundus and accelerated gastric transit *in vivo*. *Neurogastroenterol. Motil.* **28**: 827–836. [Medline] [CrossRef]
- Azuma, Y. T., Matsuo, Y., Kuwamura, M., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A. J., Nakajima, H., Karow, M. and Takeuchi, T. 2010. Interleukin-19 protects mice from innate-mediated colonic inflammation. *Inflamm. Bowel Dis.* **16**: 1017–1028. [Medline] [CrossRef]
- Briejer, M. R. and Schuurkes, J. A. 1996. 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors and cholinergic and tachykinergic neurotransmission in the guinea-pig proximal colon. *Eur. J. Pharmacol.* **308**: 173–180. [Medline] [CrossRef]
- Fujimoto, Y., Hayashi, S., Azuma, Y. T., Mukai, K., Nishiyama, K., Kita, S., Morioka, A., Nakajima, H., Iwamoto, T. and Takeuchi, T. 2016. Overexpression of  $\text{Na}^{+}/\text{Ca}^{2+}$  exchanger 1 display enhanced relaxation in the gastric fundus. *J. Pharmacol. Sci.* **132**: 181–186. [Medline] [CrossRef]



8. Gotoh, Y., Kita, S., Fujii, M., Tagashira, H., Horie, I., Arai, Y., Uchida, S. and Iwamoto, T. 2015. Genetic knockout and pharmacologic inhibition of NCX2 cause natriuresis and hypercalciuria. *Biochem. Biophys. Res. Commun.* **456**: 670–675. [[Medline](#)] [[CrossRef](#)]
9. Iwamoto, T., Pan, Y., Nakamura, T. Y., Wakabayashi, S. and Shigekawa, M. 1998. Protein kinase C-dependent regulation of Na<sup>+</sup>/Ca<sup>2+</sup> exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. *Biochemistry* **37**: 17230–17238. [[Medline](#)] [[CrossRef](#)]
10. Izzo, A. A., Gaginella, T. S. and Capasso, F. 1996. The osmotic and intrinsic mechanisms of the pharmacological laxative action of oral high doses of magnesium sulphate. Importance of the release of digestive polypeptides and nitric oxide. *Magnes. Res.* **9**: 133–138. [[Medline](#)]
11. Matsuo, Y., Azuma, Y. T., Kuwamura, M., Kuramoto, N., Nishiyama, K., Yoshida, N., Ikeda, Y., Fujimoto, Y., Nakajima, H. and Takeuchi, T. 2015. Interleukin 19 reduces inflammation in chemically induced experimental colitis. *Int. Immunopharmacol.* **29**: 468–475. [[Medline](#)] [[CrossRef](#)]
12. Nishiyama, K., Morioka, A., Kita, S., Nakajima, H., Iwamoto, T., Azuma, Y. T. and Takeuchi, T. 2014. Na/Ca(2+) exchanger 1 transgenic mice display increased relaxation in the distal colon. *Pharmacology* **94**: 230–238. [[Medline](#)] [[CrossRef](#)]
13. Nishiyama, K., Azuma, Y. T., Kita, S., Azuma, N., Hayashi, S., Nakajima, H., Iwamoto, T. and Takeuchi, T. 2013. Na<sup>+</sup>/Ca<sup>2+</sup> exchanger 1/2 double-heterozygote knockout mice display increased nitric oxide component and altered colonic motility. *J. Pharmacol. Sci.* **123**: 235–245. [[Medline](#)] [[CrossRef](#)]
14. Nishiyama, K., Azuma, Y. T., Morioka, A., Yoshida, N., Teramoto, M., Tanioka, K., Kita, S., Hayashi, S., Nakajima, H., Iwamoto, T. and Takeuchi, T. 2016. Roles of Na<sup>(+)</sup>/Ca<sup>(2+)</sup> exchanger isoforms NCX1 and NCX2 in motility in mouse ileum. *Naunyn Schmiedebergs Arch. Pharmacol.* **389**: 1081–1090. [[Medline](#)] [[CrossRef](#)]
15. Rivière, P. J., Farmer, S. C., Burks, T. F. and Porreca, F. 1991. Prostaglandin E2-induced diarrhea in mice: importance of colonic secretion. *J. Pharmacol. Exp. Ther.* **256**: 547–552. [[Medline](#)]
16. Saito, T., Mizutani, F., Iwanaga, Y., Morikawa, K. and Kato, H. 2002. Laxative and anti-diarrheal activity of polycarbophil in mice and rats. *Jpn. J. Pharmacol.* **89**: 133–141. [[Medline](#)] [[CrossRef](#)]
17. Tuladhar, B. R., Costall, B. and Naylor, R. J. 1996. 5-HT3 and 5-HT4 receptor-mediated facilitation of the emptying phase of the peristaltic reflex in the marmoset isolated ileum. *Br. J. Pharmacol.* **117**: 1679–1684. [[Medline](#)] [[CrossRef](#)]
18. Wakimoto, K., Kobayashi, K., Kuro-O, M., Yao, A., Iwamoto, T., Yanaka, N., Kita, S., Nishida, A., Azuma, S., Toyoda, Y., Omori, K., Imahie, H., Oka, T., Kudoh, S., Kohmoto, O., Yazaki, Y., Shigekawa, M., Imai, Y., Nabeshima, Y. and Komuro, I. 2000. Targeted disruption of Na<sup>+</sup>/Ca<sup>2+</sup> exchanger gene leads to cardiomyocyte apoptosis and defects in heartbeat. *J. Biol. Chem.* **275**: 36991–36998. [[Medline](#)] [[CrossRef](#)]
19. Wardle, T. D., Hall, L. and Turnberg, L. A. 1993. Inter-relationships between inflammatory mediators released from colonic mucosa in ulcerative colitis and their effects on colonic secretion. *Gut* **34**: 503–508. [[Medline](#)] [[CrossRef](#)]