Role of the vasopressin $V_1$ receptor in regulating the epithelial functions of the guinea pig distal colon

YOSHIHIKO SATO, HIROYUKI HANAI, ATSUIRO NOGAKI, KOKI HIRASAWA, EIZO KANEKO, HISAYOSHI HAYASHI, and YUICHI SUZUKI. Role of the vasopressin $V_1$ receptor in regulating the epithelial functions of the guinea pig distal colon. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G819–G828, 1999.—Vasopressin has a wide spectrum of biological action. In this study, the role of vasopressin in regulating electrolyte transport in the colon was elucidated by measuring the short-circuit current ($I_{sc}$) as well as the Na$^+$, K$^+$, and Cl$^-$ flux in a chamber-mounted mucosal sheet. The cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]) was also measured in fura 2-loaded cells by fluorescence imaging. Serosal vasopressin decreased $I_{sc}$ at $10^{-8}$ M and increased $I_{sc}$ at $10^{-7}$–$10^{-6}$ M. The decrease in $I_{sc}$ was accompanied by two effects: one was a decrease in the amiloride-sensitive Na$^+$ absorption, whereas the other was an increase in the bumetanide-sensitive K$^+$ secretion. The increase in $I_{sc}$ was accompanied by an increase in the Cl$^-$ secretion that can be inhibited by serosal bumetanide or mucosal diphenylamine 2-carboxylate. Vasopressin caused an increase in [Ca$^{2+}$]i in crypt cells. These responses of $I_{sc}$ and the [Ca$^{2+}$]i increase in crypt cells were all more potently inhibited by the vasopressin $V_1$ receptor antagonist than by the $V_2$ receptor antagonist. These results suggest that vasopressin inhibits electrogenic Na$^+$ absorption and stimulates electrogenic K$^+$ secretion. In all of these responses, the $V_1$ receptor is involved, and the [Ca$^{2+}$]i increase may play an important role.

sodium absorption; potassium secretion; intracellular calcium; intestinal transport

VASOPRESSIN PLAYS a major role in controlling the whole body water and electrolyte homeostasis (35). Vasopressin also has many other actions, such as contraction of the vascular smooth muscle and glycogenolysis in hepatocytes (42). Two kinds of receptor subtypes specific to vasopressin, i.e., the $V_1$ receptor and $V_2$ receptor, have been demonstrated to be present on the plasma membrane. Stimulation of the $V_1$ receptor leads to a rise in cytosolic Ca$^{2+}$, whereas stimulation of the $V_2$ receptor induces a rise in the cytosolic cAMP level (20, 31, 42).

The colon, the terminal part of the gastrointestinal tract, performs both the absorption and secretion of a variety of electrolytes by the epithelial transport systems. These transport systems are regulated by many kinds of endocrine, neurocrine, and paracrine agents and probably play an important role in maintaining fluid and electrolyte homeostasis in the whole body (3). It has been shown that vasopressin can affect electrolyte and water transport in the colon (37). For example, vasopressin has been reported to inhibit Na$^+$ and Cl$^-$ absorption in vivo in the rat and human colon (7, 26). In contrast, with in vitro preparations of the mouse, rat, and human colon, the stimulation of NaCl and water absorption, as well as the inhibition of Cl$^-$ secretion by vasopressin, have been demonstrated (2, 4, 5, 13, 14, 22, 44). Therefore, these previous findings are contradictory, even within the same species. Because the interpretation of the in vivo experimental results could be complicated by vasopressin action not only on the mucosal transport function but also on the microcirculation (42) and motility (36), further experiments with isolated mucosae or epithelial cells would be required to define the effect of vasopressin on electrolyte transport in the colon. In addition, the cellular mechanisms for the action of vasopressin, including the receptor subtype and the intracellular second messenger, are largely unknown.

The aim of the present study was to further characterize the role of arginine vasopressin (AVP) in the regulation of electrolyte transport in the mammalian distal colon. To this end, we measured the short-circuit current ($I_{sc}$) and ion flux in isolated guinea pig distal colon mounted in Ussing chambers. We used this method to assess the specific effects of AVP on the following three electrogenic ion transport systems: 1) electrogenic Na$^+$ absorption, 2) electrogenic K$^+$ secretion, and 3) electrogenic Cl$^-$ secretion (3, 33). We also tested which of the vasopressin receptor subtypes ($V_1$ vs. $V_2$) was involved in those effects by using vasopressin receptor antagonists. Furthermore, we assessed whether AVP could increase the cytosolic free Ca$^{2+}$ concentration ([Ca$^{2+}$]) in crypt cells with fluorescence imaging.

METHO

Tissue preparation. Male Hartley guinea pigs weighing 300–500 g were used in the experiments. All animals were fed ad libitum on a standard diet (type GM-1, Funabashi Farm, Chiba, Japan) and had free access to water until the time of the experiments. The animals were then stunned by a blow to the head and bled to death. A 10-cm segment of the distal colon was obtained from 5 cm proximal to the anus. The colon was opened longitudinally into a flat sheet, and the mucosa was separated from the underlying connective tissue and muscle with glass microscope slides (46). Histological studies revealed that the plane of division was between the muscularis mucosa and the submucosa (data not shown). In several experiments, animals were injected twice with 375

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µg/kg body wt of aldosterone (1.39 mM in saline) to enhance the electric field, once by a subcutaneous injection the evening before the experiment, and then by an intramuscular injection 4 h before the start of the experiment (referred to as the aldosterone-treated animals). Unless otherwise indicated, the experiments were conducted on animals without the aldosterone treatment.

Measurement of the electrical parameters. I\(_{\text{sc}}\) and transepithelial conductance (G\(_{\text{t}}\)) were measured as previously described (46). Stripped mucosa was mounted vertically between Ussing-type chambers with an internal surface area of 0.5 cm\(^2\). The volume of the bathing solution in each chamber was 10 ml, and its temperature was kept at 37°C in a water-jacketed reservoir. The bathing solution contained (in mM) 119 NaCl, 21 NaHCO\(_3\), 2.4 K\(_2\)HPO\(_4\), 0.6 K\(_3\)PO\(_4\), 1.2 CaCl\(_2\), 1.2 MgCl\(_2\), and 10 glucose. To provide a high-K\(^+\) solution, 100 mM KCl was used in place of 100 mM NaCl (total K\(^+\) concentration of 105.4 mM), whereas a low-Ca\(^2+\) solution was prepared by omitting CaCl\(_2\) and adding 0.2 mM gluconate. A Cl\(^-\)-free solution was made with 119 mM sodium-glucuronate, 1.2 mM magnesium-(glucuronate), and 8 mM calcium-(glucuronate), in place of 119 mM NaCl, 1.2 mM MgCl\(_2\), and 1.2 mM CaCl\(_2\), respectively. Each solution was gassed with 95% O\(_2\)-5% CO\(_2\), pH 7.4. I\(_{\text{sc}}\) values were measured with an automated voltage-clamping device (CEZ9100; Nihon Kohden, Tokyo, J apan), the value being referred to as positive when the current flowed from the mucosa to the serosa. The transepithelial potential was measured through 1 M KCl agar bridges connected to a pair of calomel half-cells. The transepithelial current was applied across the tissue via a pair of Ag-AgCl electrodes kept in contact with the mucosal and serosal bathing solutions, a pair of 1 M NaCl agar bridges being used, except when the Cl\(^-\)-flux was determined (see Measurement of the Na\(^+\), K\(^+\), and Cl\(^-\)-flux). G\(_{\text{t}}\) was calculated from the change in current in response to periodic (every 1 min) voltage pulses according to Ohm's law.

Measurement of the Na\(^+\), K\(^+\), and Cl\(^-\)-flux. The transepithelial bidirectional flux of both \(^{22}\)Na\(^+\) and \(^{42}\)K\(^+\) was determined simultaneously under short-circuit conditions. The mucosal-to-serosal and serosal-to-mucosal fluxes were measured in adjacent tissues that had G\(_{\text{t}}\) values differing within 30%. Thirty minutes was allowed for the isotopic steady state to be reached after the bathing solution on one side of the tissue had been labeled with both \(^{22}\)NaCl and \(^{42}\)KCl. Six samples (0.5-ml each) were then taken from the unlabeled side at 10-min intervals, each being replaced with an equal volume of the unlabeled solution. AVP was added immediately after 20 min of basal flux measurements. \(^{42}\)K\(^+\) was assayed immediately with a Packard AutoGamma counter (model 5650), and \(^{22}\)Na\(^+\) was assayed with a Beckman scintillation counter (model LS 8000) after at least 7 days had passed to allow \(^{42}\)K\(^+\) (half-life of 12.5 h) to decay. The transepithelial Cl\(^-\)-flux was determined only from the serosa to mucosa. The mucosal side was bathed with a Cl\(^-\)-free solution, whereas a normal solution was used for the serosal side, and the change in Cl\(^-\)-concentration of the mucosal solution was measured by ion-exchange chromatography. Six samples (0.1-ml each) were taken from the mucosal side at 10-min intervals. AVP was added after 20 min of basal flux measurements. Each experiment was performed while the transepithelial potential was clamped at 0 mV. K\(_2\)SO\(_4\) (0.5 M) agar bridges were used for potential measurements, and 0.5 M sodium-glucuronate agar bridges were used for current application. The difference in liquid junction potential developed at the interface of the potential bridge was ~9 mV between serosal control solution and mucosal Cl\(^-\)-free solution, this being determined by utilizing a saturated KCl-flowing bridge. This means that, under the 0-mV-clamped conditions, the transepithelial potential was actually clamped at 9 mV with mucosal-side negativity. This potential was not corrected for, because mucosal-side negativity would presumably have suppressed passive Cl\(^-\) secretion more than active Cl\(^-\) secretion, whereby changes in the level of active components would be determined more accurately because of less contamination of the passive components. Accordingly, this specific experiment was performed under the "apparent short-circuit condition."

The Cl\(^-\)-concentration of the collected mucosal fluids was determined with the use of an anion-exchange column (Shim-pack IC-A1; Shimadzu, Tokyo, J apan) and a conductivity detector (CDD-6A; Shimadzu). A 20-µl amount of a sample was injected and eluted at a flow rate of 2.5 ml/min with a mobile phase of 2.5 mM pthalic acid plus 2.4 mM Tris.

Measurement of [Ca\(^{2+}\)], [Ca\(^{2+}\)], was measured in crypt cells with fura 2 and fluorescence imaging. Colonic crypts were obtained as previously described (8). Small fragments of mucosa were incubated for 10 min at 37°C in a Ca\(^{2+}\)-free solution containing 0.1% BSA. Fura 2-AM (5-10 µM) was also added to the solution. The fura 2-loaded mucosal fragments were then vibrated manually in the Ca\(^{2+}\)-free solution to separate intact crypts. The Ca\(^{2+}\)-free solution contained (in mM) 107 NaCl, 5 KCl, 1 CaCl\(_2\), 0.8 MgCl\(_2\), 10 HEPES, 5 glucose, and 2.5 l-glutamine, gassed with 95% O\(_2\)-5% CO\(_2\), pH 7.4. The isolated crypt cells loaded with fura 2 were attached to the bottom of a small chamber with Cell-Tak. This chamber was then mounted on the stage of an inverted microscope equipped for epifluorescence (Diaphot; Nikon, Tokyo, J apan), and the cells were continuously superfused with a modified Hank’s balanced salt solution containing (in mM) 137 NaCl, 5 KCl, 1 CaCl\(_2\), 0.8 MgCl\(_2\), 10 HEPES, 5 glucose, and 2.5 l-glutamine, gassed with 100% O\(_2\), pH 7.4. The Ca\(^{2+}\)-free solution used for [Ca\(^{2+}\)] measurements was prepared by omitting CaCl\(_2\) from the previous solution and adding 1 mM EGTA. The temperature of the solution in the chamber was kept at 33–34°C by prewarming it. [Ca\(^{2+}\)] of each preparation loaded with fura 2 was measured by ratiometry with the dual-wavelength excitation technique (17). Digital imaging of the fura 2 fluorescence emitted at 510 nm was carried out during alternate excitation at 340 and 380 nm with a digital image analysis system (ARGUS-100; Hamamatsu Photonics, Hamamatsu, J apan), with the use of a silicon-intensified target camera. Pairs of digital images were successively obtained at specific time intervals (mostly 5–10 s) and stored. The ratio of each image pair was calculated off line with background correction, first pixel by pixel and then continued with the mean ratio value for the area of interest. The relationship between this ratio and [Ca\(^{2+}\)] was obtained by perfusing a cell-free, fura 2-containing solution with various Ca\(^{2+}\) concentrations. Although the relationship determined in this way could be somewhat different from that in situ (inside the cell), it may not devalue the main conclusions in this report, which were based on the relative changes in [Ca\(^{2+}\)].

Materials. AVP, amiloride, and bumetanide were purchased from Sigma (St. Louis, MO). The vasopressin V\(_1\) receptor antagonist, Des-Gly\(_{1-4}\)-[D-arginine(D-lysine)-D-tyrosine][Tyr(Et\(_2\)]Leu\(_4\);Arg]
vasopressin, and V\(_2\) receptor antagonist, [1-\([\beta\text{-mercapto}-\beta\text{-cyclcopentamethylene-propionic acid}],\)-Ile\(_5\),Ile\(_6\),Arg]vasopressin, were purchased from Peninsula Laboratories (Belmont, MA). Fura 2-AM was purchased from Molecular Probes (Eugene, OR). Cell-Tak was purchased from Becton Dickinson Labware (Bedford, MA), and diphenylamine-2-carboxylate (DPC) and the other chemicals were purchased from Wako Pure Chemicals (Osaka, J apan). Each drug was added from a concentrated stock solution dissolved
in water, except DPC, bumetanide, and fura 2-AM, which were dissolved in DMSO. The final volume of DMSO in an experimental solution was always 0.1%. $^{22}$Na was purchased from Dupont NEN (Boston, MA), and $^{42}$K$^+$ was purchased from Japan Atomic Energy Research Institute (Tokyo, Japan).

Statistical analyses. Values are expressed as means ± SE, and n = number of guinea pigs. Comparisons among multiple groups were tested by applying Scheffe’s F-test to a one-way ANOVA. Comparisons between two groups were tested with the two-tailed paired or unpaired Student’s t-test. Results were considered significantly different at P < 0.05.

RESULTS

Effects of AVP on $I_{sc}$ and $G_t$. AVP applied to the serosal bathing solution caused a dose-dependent, biphasic $I_{sc}$ response in the mucosa from the guinea pig distal colon (Fig. 1): a low concentration of AVP ($10^{-9}$ M) decreased $I_{sc}$ whereas a high concentration of AVP ($10^{-7}$–$10^{-6}$ M) increased $I_{sc}$. The $I_{sc}$ change induced by $10^{-8}$ M AVP varied according to individual tissue samples. An increase in $G_t$ generally accompanied the $I_{sc}$ response (Fig. 1), but, in some tissue samples, a small $G_t$ decrease was observed at a low concentration of AVP. In contrast, AVP ($10^{-10}$–$10^{-6}$ M) applied to the mucosal solution changed neither the $I_{sc}$ nor $G_t$ value (data not shown).

The mammalian distal colon has at least three electrogenic active ion transport systems that can underlie these $I_{sc}$ changes induced by AVP, i.e., Na$^+$ absorption and K$^+$ and Cl$^-$ secretions (3, 33). Electrogenic Na$^+$ absorption involves the amiloride-sensitive Na$^+$ channel for the apical membrane step and Na$^+$-K$^+$-ATPase for the basolateral membrane step. On the other hand, electrogenic K$^+$ and Cl$^-$ secretions involve the K$^+$ and Cl$^-$ channels, respectively, for the apical membrane step and the bumetanide-sensitive Na$^+$-K$^+$-Cl$^-$ cotransporter, and Na$^+$-K$^+$-ATPase for the basolateral membrane step. We studied the effect of AVP on each of these electrogenic transport systems.

Effect on amiloride-sensitive Na$^+$ absorption. We first assessed whether AVP would affect amiloride-sensitive electrogenic Na$^+$ absorption. The data presented in this section were obtained from the aldosterone-treated animals, in which the activity of electrogenic Na$^+$ absorption had been enhanced (see METHODS), but similar results were obtained from the animals without the aldosterone injection, which exhibited a small amiloride-sensitive $I_{sc}$ (data not shown). $I_{sc}$ was measured in the presence of serosal bumetanide ($10^{-4}$ M) to exclude a large portion of the electrogenic secretions of K$^+$ and Cl$^-$ (3, 33). Under these conditions, AVP applied to the serosal solution caused only decreases in $I_{sc}$ and $G_t$ (Fig. 2A): the increases in $I_{sc}$ and $G_t$ resulting from a high concentration of AVP (cf. Fig. 1) were largely inhibited. The decreases in $I_{sc}$ and $G_t$ by AVP under these conditions were virtually removed when the electrogenic Na$^+$ absorption was mostly inhibited by
mucosal amiloride at 10⁻⁴ M (34) (Fig. 2A), suggesting that AVP could inhibit amiloride-sensitive electrogenic Na⁺ absorption (3, 33). Serosal bumetanide alone caused an increase in I(sc) and a decrease in Gt (Fig. 2A), consistent with the inhibition of ongoing electrogenic K⁺ secretion (21, 34).

Figure 2B shows that the inhibition of amiloride-sensitive I(sc) by AVP was concentration dependent with ED₅₀ = 0.8 nM, the maximal inhibition of ~70% being attained at 10⁻⁸ M AVP. The decrease in Gt induced by AVP largely paralleled the decrease in I(sc), the maximum decrease being −1.15 ± 0.18 mS/cm² (Fig. 2B).

Effects on bumetanide-sensitive K⁺ and Cl⁻ secretion. We next examined the effect of AVP on I(sc) and Gt in the presence of mucosal amiloride with the use of tissues from animals without the aldosterone treatment. Under this condition, AVP still caused a concentration-dependent biphasic I(sc) response, i.e., a decrease at low concentrations and an increase at high concentrations of AVP, these being accompanied only by an increase in Gt (Fig. 3). Both the decrease and increase in I(sc) induced by AVP, as well as the increase in Gt by AVP, were all largely inhibited by serosal bumetanide at 10⁻⁴ M (Fig. 3), suggesting that the I(sc) decrease was due to the activation of electrogenic K⁺ secretion and that the I(sc) increase was due to the activation of electrogenic Cl⁻ secretion (3, 21, 33, 34, 38, 46). In addition, when 10⁻⁹ M AVP was applied, a small transient I(sc) increase was seen before the I(sc) decrease (Fig. 3A; see also Figs. 4A and 5A). Although this I(sc) increase has not been characterized any further, it could have been due to weak activation of the electrogenic Cl⁻ secretion induced by this concentration of AVP.

To test whether the I(sc) decrease in the presence of amiloride was actually due to the activation of K⁺ secretion, we used a high-K⁺ solution ([K⁺] = 105.4 mM) as the mucosal bathing solution. Under this condition, K⁺ secretion would be suppressed as a result of the decrease in electrochemical driving force for K⁺ to pass over to the mucosal side. As shown in Fig. 4, the I(sc) response induced by 10⁻⁹ M AVP was reversed from the decrease to a small increase by the high-K⁺ mucosal solution.
solution, supporting the idea that the $I_{sc}$ decrease reflected the activation of electrogenic $K^+$ secretion. The increase in $G_t$ induced by AVP was significantly greater in the high-$K^+$ mucosal solution than in the low-$K^+$ normal mucosal solution. In contrast to the response to $10^{-9}$ M AVP, the magnitude of the increases in $I_{sc}$ and $G_t$ induced by cumulatively added $10^{-8}$ M AVP above the level in the presence of $10^{-9}$ M AVP was not significantly different between the normal and high-$K^+$ mucosal solutions: the $I_{sc}$ increases in the normal and high-$K^+$ solutions were 81 ± 24 and 132 ± 30 µA/cm² (n = 5), respectively, and the $G_t$ increases were 3.1 ± 0.9 and 4.2 ± 1.6 mS/cm², respectively.

To test whether the increase in $I_{sc}$ induced by a high concentration of AVP was the result of the activation of electrogenic $Cl^-$ secretion, we examined the effect of DPC, a chloride channel blocker (1, 6), on the AVP-induced $I_{sc}$ increase in the presence of mucosal amiloride (Fig. 5). We found that mucosal DPC (30 µM) hardly affected the $I_{sc}$ decrease from a low concentration of AVP but inhibited most of the $I_{sc}$ increase induced by a high concentration of AVP. The increase in $G_t$ induced by AVP was slightly, but significantly, reduced by DPC. These results support the proposition that an $I_{sc}$ increase due to a high concentration of AVP reflected the activation of electrogenic $Cl^-$ secretion. DPC alone slightly increased $I_{sc}$ and decreased $G_t$ ($\Delta I_{sc} = 9.1 ± 1.2$ µA/cm², $\Delta G_t = 1.8 ± 1.2$ mS/cm², n = 4).

Na+, K+, and Cl- flux measurements. The foregoing results suggest that the $I_{sc}$ responses to AVP (Fig. 1) can be explained by the inhibition of Na+ absorption and activation of K+ and Cl- secretion. To confirm the foregoing hypothesis for an ionic basis to the changes in electrical parameters induced by AVP, we determined the effect of AVP on bidirectional 22Na+ and 42K+ flux under the short-circuit condition (Table 1). Aldosterone-treated animals were used. AVP (10−7 M) decreased net 22Na+ absorption mainly due to a decrease in mucosal-to-serosal 22Na+ flux, with little change in serosal-to-mucosal 22Na+ flux. In addition, AVP stimulated net 42K+ secretion mainly due to the increase in serosal-to-mucosal 42K+ flux, with a small decrease in mucosal-to-serosal 42K+ flux. These changes in flux were accompanied by a decrease in $I_{sc}$. The magnitude of the $I_{sc}$ decrease (3.9 µmol·cm−2·h−1) was, however, not large enough to account for the sum of the changes in net 22Na+ absorption (4.0 µmol·cm−2·h−1) and net 42K+ secretion (1.5 µmol·cm−2·h−1). This discrepancy may be explained by a partial offset of $I_{sc}$ decrease due to the concomitant activation of electrogenic Cl− secretion.

<table>
<thead>
<tr>
<th>Flux, µmol·cm−2·h−1</th>
<th>J Na, m−s</th>
<th>J Na, n−m</th>
<th>J Na, net</th>
<th>J K, m−s</th>
<th>J K, n−m</th>
<th>J K, net</th>
<th>Basal</th>
<th>+AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol·cm−2·h−1</td>
<td>9.1±0.6</td>
<td>2.1±0.4</td>
<td>7.1±0.7</td>
<td>1.3±0.1</td>
<td>16±0.3</td>
<td>-0.3±0.3</td>
<td>88±9</td>
<td>88±9</td>
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</table>

Table 1. Effects of AVP on the bidirectional 22Na and 42K flux under short-circuit conditions

Values are means ± SE (n = 4). The animals used in these experiments were treated with aldosterone (see METHODS). Basal values were obtained by averaging values of initial 2 10-min flux periods. Arginine vasopressin (AVP; 10−7 M) was added to serosal bathing solution immediately after measuring basal values, and mean values of following 3 10-min flux periods were obtained. 22Na+ flux and 42K+ flux were determined simultaneously for same tissue. Time-dependent decrease in basal absorption rate by similar preparation was <10% for both 22Na+ and 42K+ flux during same experimental period (Ref. 41 and Y. Suzuki, unpublished observations). $J_{m−s}$, mucosal-to-serosal flux; $J_{s−m}$, serosal-to-mucosal flux; $J_{net}$, $J_{m−s}−J_{s−m}$, $I_{sc}$, short-circuit current; $G_t$, transepithelial conductance. *0.01 < P < 0.05; **P < 0.01 compared with basal value.
We also determined the effect of AVP on Cl⁻ flux from serosa to mucosa by ion-exchange chromatography under the apparent short-circuit condition (see Methods) (Table 2). AVP (10⁻⁶ M) significantly increased Cl⁻ flux from the serosa to mucosa in association with the increase in apparent $I_{sc}$. The increase in apparent $I_{sc}$ induced by AVP was ~60% larger than the change in Cl⁻ flux. One possible explanation for this discrepancy is that electronegatic HCO₃⁻ secretion was also activated by AVP.

AVP receptor subtypes involved in regulating ion transport. We next examined which vasopressin receptor subtype, the V₁ or V₂ receptor, would mediate the effects of AVP on the electrogenic transport systems in the colon with the V₁ antagonist and the V₂ antagonist (Table 3) (29, 30). In this and the next sections, an AVP concentration of 10⁻⁹ M was used for evaluating the K⁺ secretion, of 10⁻⁸–10⁻⁷ M for Na⁺ absorption, and of 10⁻⁶ M for Cl⁻ secretion, so that, together with pharmacological agents, the $I_{sc}$ component for each transport pathway could be separately determined (still responses are at or close to the maximum level). The reduction of electronegatic Na⁺ absorption and the enhancement of electrogenic K⁺ and Cl⁻ secretion induced by AVP were all inhibited more potently by the V₁ than by the V₂ antagonist. These antagonists had no effect on the baseline $I_{sc}$ and Gₛ levels.

Influence of the serosal low-Ca²⁺ solution on the $I_{sc}$ response to AVP. Ca²⁺ has been suggested to be an important intracellular second messenger for vasopressin V₁ receptor activation (20, 42). To study the role of Ca²⁺ in the regulation of colonic ion transport by AVP, we first examined the effect of the serosal low-Ca²⁺ solution (nominally Ca²⁺ free, containing 0.2 mM EGTA) on AVP-induced changes to the electrogenic ion transport systems (Table 4). The stimulation of K⁺ and Cl⁻ secretion by AVP were both almost completely inhibited when Ca²⁺ was removed from the serosal solution. On the other hand, serosal Ca²⁺ removal had no effect on the inhibition of electronegatic Na⁺ absorption (in terms of the percent inhibition) induced by AVP. It was noticed, however, that the magnitude of reduction of absorption.

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Δ$I_{sc}$, µA·cm⁻²</th>
<th>Inhibition of Amiloride-Sensitive $I_{sc}$, %</th>
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<tr>
<td>A. Inhibition of Na⁺ absorption</td>
<td></td>
<td></td>
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<tr>
<td>10⁻⁸ M AVP</td>
<td>−75.5 ± 18.4</td>
<td>49.6 ± 6.1</td>
</tr>
<tr>
<td>10⁻⁸ M AVP + low Ca²⁺</td>
<td>−47.7 ± 9.9</td>
<td>51.3 ± 4.6</td>
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<tr>
<td>B. Stimulation of K⁺ secretion</td>
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<td></td>
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<tr>
<td>10⁻⁹ M AVP</td>
<td>−50.0 ± 8.6</td>
<td>53.4 ± 3.8</td>
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<tr>
<td>10⁻⁹ M AVP + low Ca²⁺</td>
<td>−4.6 ± 3.3</td>
<td>51.3 ± 4.6</td>
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<tr>
<td>C. Stimulation of Cl⁻ secretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁸ M AVP</td>
<td>156.4 ± 31.4</td>
<td>51.3 ± 4.6</td>
</tr>
<tr>
<td>10⁻⁹ M AVP + low Ca²⁺</td>
<td>12.4 ± 2.0**</td>
<td>53.4 ± 3.8</td>
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Values are means ± SE (n = 4). In each series of experiments, effect of serosal AVP was examined in absence and presence of V₁ or V₂ receptor antagonist (applied to the serosal side 15 min before adding AVP) by using 3 adjacent tissues. Des-Gly[phenylacetic acid]-noprestin (AVP) was used as V₁ receptor antagonist, and I-[1-(β-mercaptopo,β-cyclopentamethylene-propionic acid),pro-lle2,Ile4,Arg8]vasopressin was used as V₂ receptor antagonist. Δ$I_{sc}$ represents magnitude of change in $I_{sc}$ due to AVP under each condition. A: To determine receptor subtype involved in inhibition of electronegatic Na⁺ absorption, $I_{sc}$ decrease by 10⁻⁸ M AVP was measured in presence of serosal bumetanide (10⁻⁶ M). Amiloride (10⁻⁴ M) was then added to mucosal side before calculating percent inhibition of amiloride-sensitive $I_{sc}$. These data were obtained from aldosterone-treated animals. B: To determine receptor subtype involved in stimulation of K⁺ secretion, $I_{sc}$ increase by 10⁻⁹ M AVP was measured in presence of mucosal amiloride (10⁻⁶ M). C: To determine receptor subtype involved in stimulation of Cl⁻ secretion, $I_{sc}$ increase by 10⁻⁹ M AVP was measured in presence of mucosal amiloride. *0.01 < P < 0.05; **P < 0.01 compared with control for each series of experiments.

<table>
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<tr>
<th>Experimental Condition</th>
<th>Δ$I_{sc}$, µA·cm⁻²</th>
<th>Inhibition of Amiloride-Sensitive $I_{sc}$, %</th>
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<tbody>
<tr>
<td>A. Inhibition of Na⁺ absorption</td>
<td></td>
<td></td>
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<tr>
<td>10⁻⁸ M AVP</td>
<td>−56.8 ± 45.0</td>
<td>78.8 ± 4.4</td>
</tr>
<tr>
<td>10⁻⁸ M AVP + 10⁻⁶ M V₂ antagonist</td>
<td>−168.8 ± 19.4</td>
<td>70.2 ± 2.0</td>
</tr>
<tr>
<td>10⁻⁹ M AVP + 10⁻⁶ M V₂ antagonist</td>
<td>−27.2 ± 5.8**</td>
<td>10.6 ± 2.6**</td>
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</tbody>
</table>

Values are means ± SE (n = 5). Mucosal side was bathed with Cl⁻-free solution, and serosal side was bathed with normal solution. Cl⁻ appearance initially Cl⁻-free mucosal solution was determined by ion-exchange chromatography. Experiments were done under "apparent short-circuit" condition, because difference in liquid junction potential between mucosal and serosal potential-measuring bridges was not corrected (see Methods). Basal $J_{cl}$ values were obtained by averaging values of initial 2 10-min flux periods. Mean $J_{cl}$ values of following 3 10-min flux periods were obtained in absence (basal) or presence of AVP (serosal, 10⁻⁶ M). $ΔI_{sc}$ and $ΔGₛ$ represent maximum changes in apparent $I_{sc}$ and Gₛ induced by AVP. This measurement employed 10⁻⁶ M of AVP rather than 10⁻⁷ M because AVP appeared to be less potent for activating Cl⁻ secretion than for activating K⁺ secretion and inhibiting Na⁺ absorption. *0.01 < P < 0.05; **P < 0.01 compared with basal value for each series of experiments.

Table 3. Effects of the vasopressin V₁ and V₂ receptor antagonists on the AVP-induced $I_{sc}$ response

Table 4. Effect of the serosal low-calcium solution on the AVP-induced $I_{sc}$ change

Table 2. Effects of AVP on the Cl⁻ flux from serosa to mucosa

$$J_{sc}$$ (µmol·cm⁻²·h⁻¹), $ΔI_{sc}$ (µA·cm⁻²), $ΔGₛ$ (mS·cm⁻²)

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Δ$I_{sc}$, µA·cm⁻²</th>
<th>Inhibition of Amiloride-Sensitive $I_{sc}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$+$ Basal</td>
<td>5.05 ± 0.86</td>
<td>−8.8 ± 9.9</td>
</tr>
<tr>
<td>$+$ Basal</td>
<td>5.36 ± 0.78</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>$+$ AVP, 10⁻⁹ M</td>
<td>4.09 ± 0.75</td>
<td>43.8 ± 41</td>
</tr>
<tr>
<td>$+$ AVP, 10⁻⁶ M</td>
<td>5.97 ± 0.5**</td>
<td>4.2 ± 1.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). Mucosal side was bathed with Cl⁻-free solution, and serosal side was bathed with normal solution. Cl⁻ appearance initially Cl⁻-free mucosal solution was determined by ion-exchange chromatography. Experiments were done under "apparent short-circuit" condition, because difference in liquid junction potential between mucosal and serosal potential-measuring bridges was not corrected (see Methods). Basal $J_{cl}$ values were obtained by averaging values of initial 2 10-min flux periods. Mean $J_{cl}$ values of following 3 10-min flux periods were obtained in absence (basal) or presence of AVP (serosal, 10⁻⁶ M). $ΔI_{sc}$ and $ΔGₛ$ represent maximum changes in apparent $I_{sc}$ and Gₛ induced by AVP. This measurement employed 10⁻⁶ M of AVP rather than 10⁻⁷ M because AVP appeared to be less potent for activating Cl⁻ secretion than for activating K⁺ secretion and inhibiting Na⁺ absorption. *0.01 < P < 0.05; **P < 0.01 compared with basal value for each series of experiments.

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amiloride-sensitive $I_{sc}$ was somewhat smaller under the serosal low-Ca$^{2+}$ condition than under the control condition. This was because of a reduced basal level of amiloride-sensitive $I_{sc}$ under the serosal low-Ca$^{2+}$ condition (data not shown), the reason for which is unknown at present. We could not assess the effect of removing Ca$^{2+}$ from both the mucosal and serosal solutions, because $G_{t}$ was greatly increased under those conditions.

Effect of AVP on [Ca$^{2+}$], in crypt cells. We next measured microfluorometrically by fluorescence imaging the changes in [Ca$^{2+}$], induced by AVP in colonic crypt cells. In the crypt cells, the baseline [Ca$^{2+}$] value was 90 ± 4 nM (n = 4). AVP (10$^{-7}$ M) increased [Ca$^{2+}$], in most portions of the crypt (Fig. 6). The increase in [Ca$^{2+}$] reached a maximum within 30 s after the application of AVP, the value being substantially sustained thereafter (Fig. 6D). When tissues were superfused with the Ca$^{2+}$-free solution (nominally Ca$^{2+}$ free, containing 1 mM EGTA), the baseline [Ca$^{2+}$] value stabilized within 1 min to 67 ± 12 nM (n = 4). Under such a Ca$^{2+}$-free condition, AVP still evoked an increase in [Ca$^{2+}$], the initial peak being little, although significantly, reduced ($\Delta$[Ca$^{2+}$]; control = 153 ± 9 nM, Ca$^{2+}$-free = 96 ± 7 nM; P < 0.01, n = 4), whereas the decline during the late phase was accelerated ($\Delta$[Ca$^{2+}$], 3 min after the peak/\Delta[Ca$^{2+}$] at the peak: control = 0.72 ± 0.11, Ca$^{2+}$-free = 0.20 ± 0.03; P < 0.05, n = 4). This result suggests that the [Ca$^{2+}$] increase induced by AVP in the crypt cells included both a Ca$^{2+}$ release from the intracellular store and a Ca$^{2+}$ influx from the extracellular fluid.

The relationship between the AVP concentration and the peak increase in [Ca$^{2+}$] ($\Delta$[Ca$^{2+}$]) was determined in the crypt cells (Fig. 7). The maximal $\Delta$[Ca$^{2+}$] value was 160 ± 5 nM, ED$_{50}$ being ~2 nM. $\Delta$[Ca$^{2+}$] by 10$^{-7}$ M AVP was inhibited more potently by the V$_1$ antagonist than by the V$_2$ antagonist (Fig. 7B), suggesting that AVP increased [Ca$^{2+}$], in the crypt cells by activating the V$_1$ receptor.

**DISCUSSION**

This study has shown that serosal AVP could regulate the three kinds of electrogenic ion transport system in the guinea pig distal colon. First, AVP inhibits electrogenic Na$^+$ absorption. The evidence for this is
that, in the presence of bumetanide to inhibit K\(^+\) and Cl\(^-\) secretions, AVP evoked a decrease in \(I_{\text{sc}}\), in association with a \(G_i\) decrease, both of these effects being absent in the presence of mucosal 10\(-4\) M amiloride (Fig. 2) (3, 33). In addition, AVP reduced \(^{22}\text{Na}\)\(^+\) absorption (Table 1). It is unlikely that the reduction of \(^{22}\text{Na}\)\(^+\) absorption induced by AVP (4 \(\mu\)mol \cdot cm\(^{-2}\) \cdot h\(^{-1}\)) was due mainly to the inhibition of electroneutral Na\(^+\) absorption rather than the inhibition of the electronegative Na\(^+\) absorption because, in the aldosterone-treated guinea pig distal colon, the rate of electronegative Na\(^+\) absorption (estimated from the rate of \(^{22}\text{Na}\)\(^+\) absorption) in the presence of 10\(-4\) M mucosal amiloride was <2 \(\mu\)mol \cdot cm\(^{-2}\) \cdot h\(^{-1}\) (T. Yamamoto and Y. Suzuki, unpublished observations). The maximum inhibition of electronegative Na\(^+\) absorption by AVP was ~70%. Second, AVP stimulates electrogenic K\(^+\) secretion. When the electrogenic Na\(^+\) absorption was inhibited by amiloride, AVP at a low concentration (10\(-9\) M) evoked a decrease in \(I_{\text{sc}}\) associated with an increase in \(G_i\) (Fig. 3). This \(I_{\text{sc}}\) decrease was inhibited by serosal bumetanide, an inhibitor of the Na\(^+\)\(-\text{K}\(^+\))\(-\text{Cl}\(^-\)) cotransporter, or when the high-K\(^+\) solution was used for the mucosal solution (Figs. 3 and 4). These results are consistent with the notion of AVP stimulating electrogenic K\(^+\) secretion by AVP (Table 1). Third, AVP stimulates electrogenic Cl\(^-\) secretion. The evidence for this is that AVP at a relatively high concentration (~10\(-7\) M) increased \(I_{\text{sc}}\), which was inhibited by bumetanide (Fig. 3). This \(I_{\text{sc}}\) increase, when corrected for the \(I_{\text{sc}}\) decrease due to K\(^+\) secretion, was not noticeably influenced by the high-K\(^+\) mucosal solution, but was attenuated by mucosal DPC, a Cl\(^-\) channel-blocking acrylamidobenzoate (1, 6) (Figs. 4 and 5). These findings are consistent with the activation of electrogenic Cl\(^-\) secretion (3, 33, 34, 46). The result of the Cl\(^-\) flux study confirmed the activation of K\(^+\) secretion by AVP (Table 1). Collectively, the present results strongly suggest that AVP would inhibit electrogenic Na\(^+\) absorption and stimulate electrogenic K\(^+\) and Cl\(^-\) secretions. It cannot be excluded, however, that AVP could also regulate such other ion-transport systems demonstrated in the colon as Na\(^+\) absorption mediated by Na\(^+\)/H\(^+\) exchange, Cl\(^-\) absorption/HCO\(_3\)\(^-\) secretion mediated by Cl\(^-\)/HCO\(_3\)\(^-\) exchange, and K\(^+\) absorption/H\(^+\) secretion mediated by H\(^+\)-K\(^+\)-ATPase (3, 40). These transport systems are believed to be nonelectrogenic and thus cannot be assessed from \(I_{\text{sc}}\) measurements.

The vasopressin receptor is of two different subtypes, the V\(_1\) and V\(_2\) receptors (20, 31, 42). The present study has demonstrated that the regulation of three kinds of electrogenic ion transport in the colon by AVP was probably all mediated by the V\(_1\) receptor, because they were each inhibited more potently by the V\(_1\) than by the V\(_2\) receptor antagonist (Table 3) (29, 30). The dissociation constant for activating AVP has been reported to be 0.6–3 nM (42). The \(ED_{50}\) value for inhibiting electrogenic Na\(^+\) absorption (0.8 nM) was within this range (Fig. 2). The \(ED_{50}\) value for activating K\(^+\) secretion may also be within this range (0.1–1 nM; Figs. 3 and 5), whereas that for activating Cl\(^-\) secretion seems to be at a slightly higher value (~10 nM; Fig. 3). It cannot be excluded, however, that there is also the V\(_2\) receptor in the colonic mucosa, which is involved in regulating nonelectrogenic transport systems, such as those just described, or in modulating other physiological regulators acting on the electrogenic transport systems.

The present study has demonstrated by fluorescence imaging that the AVP receptor connected with the [Ca\(^{2+}\)]\(^+\) increase was present in crypt cells of the distal colon. The AVP receptor in crypt cells is probably of the V\(_1\) subtype, because the [Ca\(^{2+}\)]\(^+\) increase was inhibited more potently by the V\(_1\) receptor antagonist than by the V\(_2\) receptor antagonist (Fig. 7). This agrees with the general notion that the V\(_1\) receptor is coupled to the Ca\(^{2+}\) signaling pathway (20, 42). The relationship between the concentration of AVP and the magnitude of the [Ca\(^{2+}\)]\(^+\) increase in crypt cells reveals an \(ED_{50}\) value of ~2 nM (Fig. 7), well within the range for the dissociation constant of AVP for the V\(_1\) receptor (see Fig. 3). It is possible, although it remains to be demonstrated, that the V\(_1\) receptor coupled to the Ca\(^{2+}\) signaling pathways is also present in surface cells, because electrogenic Na\(^+\) absorption, which is presumed to mainly reside in surface cells (10, 24, 28), was regulated by a V\(_1\) receptor-mediated mechanism. We have attempted to prepare isolated surface cells to measure [Ca\(^{2+}\)]\(^+\) and found that AVP caused an increase in [Ca\(^{2+}\)]\(^+\) (Y. Sato, unpublished observations). However, we cannot exclude the possibility that such preparations were contaminated by cell types other than surface cells.

It is possible that both the AVP-induced [Ca\(^{2+}\)]\(^+\) increase in the crypt cells (Fig. 6) and that in the surface cells (as just proposed) are important parts of the signal transduction pathway for the V\(_1\) receptor-mediated regulation of electrogenic transport systems in the distal colon. We have shown here that the activation of Cl\(^-\) and K\(^+\) secretions induced by AVP depended on the presence of Ca\(^{2+}\) in the serosal medium (Table 4). It has been shown that Ca\(^{2+}\) is one of the most important mediators for activating Cl\(^-\) (3, 9, 46) and K\(^+\) secretions in the intestine (34, 38). Cl\(^-\) secretion in the distal colon has been suggested to occur mainly in crypt cells (18, 39), although Köckerling and Fromm (23) have demonstrated that Cl\(^-\) secretion is also considerably performed by surface cells. Thus the AVP-induced [Ca\(^{2+}\)]\(^+\) increase in crypt cells could be intimately related to the AVP-induced activation of Cl\(^-\) secretion. The Ca\(^{2+}\) increase in crypt cells has been shown to be induced by agents that can stimulate Cl\(^-\) secretion, such as muscarinic agonists and ATP (25, 27). In addition, Ca\(^{2+}\)-dependent activation of the ion channels that may underlie the activation of transepithelial Cl\(^-\) secretion has recently been reported to exist in colonic crypt cells (15). On the other hand, the cellular type responsible for electrogenic K\(^+\) secretion is equivocal: both surface and crypt cells have been suggested to be involved (16, 18, 28, 38). In contrast to
the activation of Cl⁻ and K⁺ secretions, the AVP-induced inhibition of electrogenic Na⁺ absorption was not influenced by the serosal low-Ca²⁺ solution (Table 4). However, we think that the increase in [Ca²⁺]i could also play a role here, because Ca²⁺-mobilizing agents such as a muscarinic agonist (41) or calcium ionophore A23187 (Y. Suzuki, unpublished observations) inhibited electrogenic Na⁺ absorption in the colon. The Ca²⁺-dependent inhibition of apical amiloride-sensitive Na⁺ channels has been demonstrated in other Na⁺-transporting epithelia (11, 12, 32). Because electrogenic Na⁺ absorption is not influenced by the serosal low-Ca²⁺ solution, Ca²⁺-mediated electrogenic Na⁺ absorption could be mediated by a decrease in [Ca²⁺]i in the surface cells (10, 24, 28). The possible [Ca²⁺]i increase in the surface cells could be related to the inhibition of electrogenic Na⁺ absorption by AVP. The failure to influence the effect of AVP on Na⁺ absorption by serosal Ca²⁺ removal (Table 4) might be explained by the idea that Ca²⁺ released from the intracellular Ca²⁺ store is more important than that from an extracellular source, or that Ca²⁺ entering from the mucosal side plays the major role. Accordingly, the increased [Ca²⁺]i in the crypt and surface cells might be involved in regulation of the Na⁺ absorption, Cl⁻ secretion, and K⁺ secretion by AVP. Several findings from the present study are, however, apparently inconsistent with this conclusion; for example, the ED₅₀ value for increased [Ca²⁺]i in the crypt cells by AVP was 2 nM, whereas that for Cl⁻ secretion seems to have been >10 nM (Fig. 3). Clearly, further studies will be needed to elucidate the precise role played by [Ca²⁺]i in regulating electrolyte transport by AVP in the colon.

Vasopressin has previously been reported to influence fluid and electrolyte transport in the mammalian small and large intestines. Both stimulation and inhibition of Na⁺ and water absorption have been shown (37). For example, in vivo studies on the human and rat colon have demonstrated that intravenous vasopressin administration inhibited NaCl and water absorption (7, 26), whereas K⁺ secretion was unchanged (26); this generally agrees with the present findings of the inhibition of Na⁺ absorption and the stimulation of Cl⁻ secretion by AVP in the guinea pig distal colon. On the other hand, in vitro studies on isolated human, mouse, and rat colons have reported that AVP stimulated Na⁺ absorption (2, 4, 5, 13, 14, 22, 44). It might be difficult, however, with in vivo studies to entirely exclude the possibility that those AVP-induced changes in electrolyte and fluid transport were the result of changes in the mucosal blood supply or in intestinal motility, rather than changes in epithelial transport per se (7, 26). Extensive studies on the rat colon in vitro (4, 5, 14) have shown that AVP enhanced amiloride-insensitive Cl⁻-dependent Na⁺ absorption and inhibited electrogenic Cl⁻ secretion in the distal but not in the proximal colon. In addition, AVP had no effect on electrogenic amiloride-sensitive Na⁺ absorption in the rat colon (5). We have no explanation other than species difference to explain the different effects of AVP on the rat colon, in spite of the use of a similar experimental technique. The effect of AVP on the rat colon has been suggested to be mediated by a decrease in [Ca²⁺]i (4, 22).

The circulating AVP concentration is ~1 pM in euhydrated mammals, which is elevated to ~100 pM under severe hypovolemia (35). Thus the action of plasma AVP on colonic electrolyte transport via the V₁ receptor can only be exerted under relatively severe pathophysiological conditions. Alternatively, AVP released by a neurocrine or paracrine mechanism within the mucosa could be the main factor responsible for regulating colonic ion transport (19). A recent study has demonstrated the presence of vasopressin-like immunoreactivity in epithelial, submucosal, and muscle layers ranging from the stomach to rectum (43, 45).

The physiological consequences of the changes in ion transport induced by AVP that have been demonstrated here could be lubrication of the mucosal surface layer of the colon by secreted fluid resulting from the stimulation of K⁺ and Cl⁻ secretion and the inhibition of Na⁺ absorption. These effects of AVP, in concert with the V₁ receptor-mediated contraction of smooth muscle and the stimulation of peristalsis (36), would help the smooth passage of intestinal contents. Alternatively, if the changes in ion transport by AVP are not associated with the net transepithelial water movement, the inhibition of Na⁺ absorption and stimulation of Cl⁻ and K⁺ secretion may be able to contribute to lowering the plasma osmolarity, a well-known systemic effect of AVP (35). The source of AVP responsible for regulating ion transport in the colon as well as the physiological significance of this regulation remain to be elucidated.

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Address for reprint requests and other correspondence: Yoshihiko Sato, First Dept. of Medicine, Hamamatsu Univ. School of Medicine, 3660 Handa-cho, Hamamatsu 431-3192, Japan (E-mail: ysato26@hama-med.ac.jp).

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Vasopressin V1 receptor and colonic ion transport