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Am J Physiol Cell Physiol 288:C1431-C1439, 2005. First published 19 January 2005;
doi: 10.1152/ajpcell.00373.2004

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Dual effects of *n*-alcohols on fluid secretion from guinea pig pancreatic ducts

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Submitted 30 July 2004; accepted in final form 16 January 2005

Hamada, Hiroyuki, Hiroshi Ishiguro, Akiko Yamamoto, Sachiko Shimano-Futakuchi, Shigeru B. H. Ko, Toshiyuki Yoshikawa, Hidemi Goto, Motoji Kitagawa, Tetsuo Hayakawa, Yoshiteru Seo, and Satoru Naruse. Dual effects of *n*-alcohols on fluid secretion from guinea pig pancreatic ducts. *Am J Physiol Cell Physiol* 288: C1431–C1439, 2005. First published January 19, 2005; doi:10.1152/ajpcell.00373.2004.—Ethanol strongly augments secretin-stimulated, but not acetylcholine (ACh)-stimulated, fluid secretion from pancreatic duct cells. To understand its mechanism of action, we examined the effect of short-chain *n*-alcohols on fluid secretion and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in guinea pig pancreatic ducts. Fluid secretion was measured by monitoring the luminal volume of isolated interlobular ducts. $[Ca^{2+}]_i$ was estimated using fura-2 microfluorometry. Methanol and ethanol at 0.3–10 mM concentrations significantly augmented fluid secretion and induced a transient elevation of $[Ca^{2+}]_i$ in secretin- or dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP)-stimulated ducts. However, they failed to affect fluid secretion and $[Ca^{2+}]_i$ in unstimulated and ACh-stimulated ducts. In contrast, propanol and butanol at 0.3–10 mM concentrations significantly reduced fluid secretion and decreased $[Ca^{2+}]_i$ in unstimulated ducts and in ducts stimulated with secretin, DBcAMP, or ACh. Both stimulatory and inhibitory effects of *n*-alcohols completely disappeared after their removal from the perfusate. Propanol and butanol inhibited the plateau phase, but not the initial peak, of $[Ca^{2+}]_i$ response to ACh as well as the $[Ca^{2+}]_i$ elevation induced by thapsigargin, suggesting that they inhibit Ca^{2+} influx. Removal of extracellular Ca^{2+} reduced $[Ca^{2+}]_i$ in duct cells and completely abolished secretin-stimulated fluid secretion. In conclusion, there is a distinct cutoff point between ethanol (C2) and propanol (C3) in their effects on fluid secretion and $[Ca^{2+}]_i$ in duct cells. Short-chain *n*-alcohols appear to affect pancreatic ductal fluid secretion by activating or inhibiting the plasma membrane Ca^{2+} channel.

intracellular calcium; acetylcholine

ALCOHOL IS THE LEADING CAUSE of acute and chronic pancreatitis, but few studies have succeeded in demonstrating cellular effects of ethanol that directly cause pancreatitis. Most studies have been conducted with acinar cells and the significant effect, such as a sustained increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (6), was observed at a concentration (850 mM) that far exceeded lethal concentrations (>80 mM) of ethanol in humans (16). Furthermore, these effects appear to be mediated by ethanol's nonoxidative metabolites rather than by ethanol itself (6, 28). Recently, we found that 0.3–30 mM ethanol, relevant to usual levels of alcohol drinking (21), strongly augmented secretin-stimulated fluid secretion from interlobular ducts isolated from the guinea pig pancreas (31). The pancreatic duct system produces a HCO_3^--rich fluid secretion in response to secretin and acetylcholine (ACh) via elevation of intracellular cAMP and $[Ca^{2+}]_i$, respectively (3). HCO_3^- is secreted into the lumen via apical Cl^-/HCO_3^- exchange and the cystic fibrosis transmembrane conductance regulator (CFTR) (3, 15). Water transport via the basolateral and apical water channel (aquaporin-1, AQP1) follows a small osmotic gradient caused by apical HCO_3^- transport (11, 18, 31). The augmentation of fluid secretion by ethanol appears to be mediated by the activation of the intracellular cAMP pathway and a transient increase in $[Ca^{2+}]_i$. Because very low concentrations of ethanol quickly and reversibly induced the augmentation, we speculate that ethanol directly affects some ion channels and/or transporters, such as the plasma membrane Ca^{2+} channel and the CFTR Cl^- channel, rather than altering cellular metabolism (24).

Ethanol produces profound alterations in brain function. There has been a longstanding debate concerning whether alcohols produce their effects by acting on the membrane lipids or proteins of the central nervous system (CNS) neurons (20, 25). The "lipid theory" postulates that alcohols affect primarily membrane fluidity and thereby modify the function of membrane ion channels, receptors, and other proteins. The "protein theory," on the other hand, proposes that alcohols interact directly with these membrane proteins. Accumulating evidence suggests that proteins are the primary sites of action (25). Although the primary site of action differs between the two theories, both theories attribute the effects of alcohol to alterations in protein function. The CNS effects of alcohols are notably characterized by the cutoff effect: the potency of an alcohol increases with increasing alkyl chain length until a point is reached at which the potency attains a maximum and then decreases or disappears with further increases in chain length (20). In this article, we report a distinct cutoff effect of a series of short-chain *n*-alcohols on fluid secretion and $[Ca^{2+}]_i$ in interlobular ducts isolated from the guinea pig pancreas. Our observations suggest that alcohols strongly affect the function of not only CNS neurons but also nonexcitable epithelial cells.

METHODS

The present study was approved by the Ethical Committee of Nagoya University on Animal Use for Experiments.

Materials and Solutions

Methanol, ethanol, 1-propanol, 1-butanol, ACh, dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP), thapsigargin, ethylene

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glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), and all other standard laboratory chemicals were of the highest grade available from Sigma (St. Louis, MO). The purities of alcohols were >99.5%. Fura-2 AM (Dojindo Laboratories, Kumamoto, Japan) was dissolved in dimethyl sulfoxide (DMSO), and its loading solution contained 0.1% DMSO. Secretin (Peptide Institute, Minoh, Japan) was dissolved in standard HCO_3^--buffered solution containing 0.5% bovine serum albumin (Sigma), and the final concentration of albumin in the bathing solution was 0.01%. The HEPES-buffered solution contained (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 10 HEPES (~280 mosmol/l) and was equilibrated with 100% O_2. The standard HCO_3^--buffered solution contained (in mM) 115 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 25 $NaHCO_3$ (~280 mosmol/l). The HCO_3^--buffered solution with lower $[Ca^{2+}]$ (expected to be <1 μM) contained 1 mM $CaCl_2$ and 3 mM EGTA (17). The Ca^{2+}-free, HCO_3^--buffered solution contained 5 mM EGTA but no $CaCl_2$. In these solutions, aliquots of 50 mM Na_2H_2-EGTA solution were added and NaCl concentrations were reduced to maintain the same osmolarity as the standard solution. HCO_3^--buffered solutions were equilibrated with 95% O_2-5% CO_2. All solutions were adjusted to pH 7.4 at 37°C with 1 N HCl.

Isolation and Culture of Interlobular Ducts

Female Hartley guinea pigs (~350 g) were killed by cervical dislocation. The pancreas was removed, and interlobular ducts (diameter, 100–150 μm) were isolated as described previously (14). The duct segments were cultured at 37°C in 5% CO_2 in air for 3 h, during which time both ends of the interlobular duct segments sealed spontaneously, thus isolating the luminal space from the bathing medium.

Measurement of the Fluid Secretory Rate

The fluid secretory rate into the closed luminal space was measured as we described previously (27, 31). The sealed ducts were superfused at 37°C on the stage of an inverted microscope, and the bright-field images were obtained at 1-min intervals using a charge-coupled device camera. The initial values for the length (L_0), diameter ($2R_0$), and image area (A_0) of the duct lumen were measured in the first image of the series. The initial volume (V_0) of the duct lumen was calculated as $\pi R_0^2 L_0$ assuming cylindrical geometry. The L_0, R_0, and V_0 values of all ducts used for experiments were 315 ± 12 μm, 75 ± 3 μm, and 6.0 ± 0.4 nl, respectively (n = 87; means ± SE). The luminal surface area of the epithelium was assumed to be $2\pi R_0 L_0$. In subsequent images of the series, the luminal image area (A) was expressed as relative area (A/A_0). Relative volume (V/V_0) was estimated from relative area using the equation $V/V_0 = (A/A_0)^{3/2}$. The rate of fluid secretion was calculated at 1-min intervals from the increment in volume and expressed as the secretory rate per unit of luminal area of epithelium ($nl \cdot min^{-1} \cdot mm^{-2}$).

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was estimated using microfluorometry in duct cells loaded with fura-2 as we described previously (19). The cultured duct segments were incubated for 90 min at room temperature with the fura-2 AM (3 μM). Microfluorometry was performed on a small area of the ductal epithelium containing from three to five cells illuminated alternately at 340 and 380 nm. The fluorescence intensities (F_{340} and F_{380}) were measured at 510 nm. Changes in $[Ca^{2+}]_i$ are presented as changes in the F_{340}-to-F_{380} fluorescence ratio.

Statistics

Data are presented as means ± SE, with n representing the number of ducts. Statistical analysis was performed using Student's t-test for paired data or ANOVA followed by Tukey's procedure for multiple comparisons, with $P < 0.05$ used as the level of significance.

RESULTS

Effects of n-Alcohols on Fluid Secretion

Spontaneous (HCO_3^- dependent) fluid secretion. The isolated interlobular ducts secreted little fluid in the absence of HCO_3^-, i.e., in a HEPES-buffered solution (13). When the ducts were superfused with the standard HCO_3^--buffered solution, the fluid secretory rate was 1.01 ± 0.07 $nl \cdot min^{-1} \cdot mm^{-2}$ (n = 18; Fig. 1). When 1 mM methanol or ethanol was added to the bath, fluid secretion transiently stopped for a 1-min period but quickly recovered to steady-state levels in the next 1-min period ($P < 0.01$). Bath application of 1 mM propanol or butanol significantly ($P < 0.05$) and reversibly reduced the spontaneous fluid secretion. A transient cessation in secretion was not observed.

Secretin (cAMP)-stimulated fluid secretion. To avoid rupture of the ducts that was sometimes caused by the increased

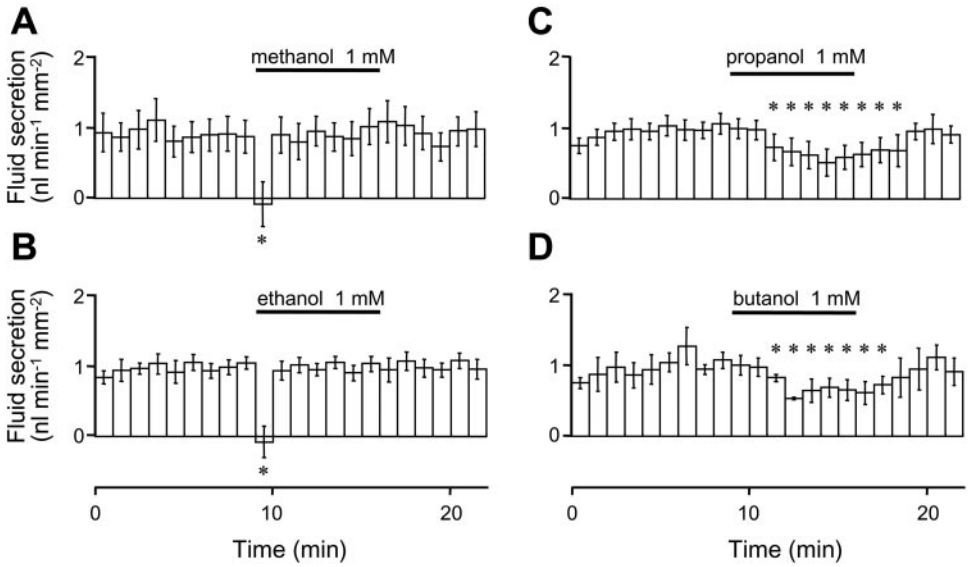

Fig. 1. Effects of *n*-alcohols on fluid secretion (mean ± SE) in unstimulated ducts. The closed ducts were superfused with the standard HCO_3^--CO_2-buffered solution. Alcohols at 1 mM were added to the bath as indicated. *A*: methanol (n = 4). *B*: ethanol (n = 5). *C*: propanol (n = 5). *D*: butanol (n = 4). *$P < 0.05$, significant difference from secretory rate before application of *n*-alcohols.

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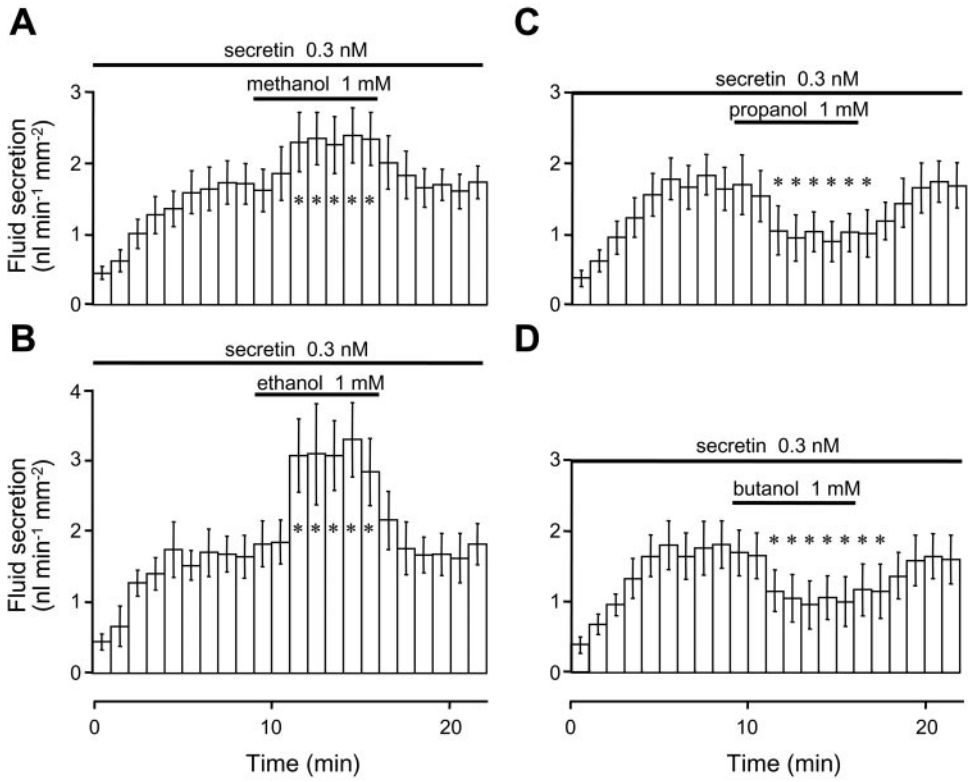

Fig. 2. Effects of *n*-alcohols on fluid secretion (means ± SE; $n = 5$) in secretin-stimulated ducts. Methanol (*A*), ethanol (*B*), propanol (*C*), or butanol (*D*) at 1 mM was added to the bath during stimulation with 0.3 nM secretin. Note that the perfusate was switched from HEPES- to HCO_3^--buffered solution at *time 0*. $*P < 0.05$, significant difference from secretory rate before application of *n*-alcohols.

luminal pressure due to accumulated fluid secretion within the closed lumen, the total experimental period needed to be <25 min. Therefore, in the following experiments (Figs. 2 and 3), the perfusate was switched from HEPES- to HCO_3^--buffered solution when the stimulants were applied. Secretin (0.3 nM) increased the fluid secretory rate from 0 to 1.67 ± 0.01 $nl \cdot min^{-1} \cdot mm^{-2}$ ($n = 20$) within ~5 min, and this rate remained constant for >15 min (Fig. 2). Methanol and ethanol at 1 mM significantly ($P < 0.01$) and reversibly increased the secretin-stimulated fluid secretion by 41 ± 7% and 85 ± 6%, respectively ($n = 5$). The augmentation by ethanol was significantly ($P < 0.01$) greater than that by methanol. Propanol and butanol (1 mM), on the other hand, significantly ($P < 0.01$) and reversibly reduced the secretin-stimulated fluid secretion by 44 ± 13% and 44 ± 9%, respectively ($n = 5$). Similarly, DBcAMP (0.5 mM), a membrane-permeable analog of cAMP, increased the fluid secretory rate to 1.49 ± 0.11 $nl \cdot min^{-1} \cdot mm^{-2}$ ($n = 18$). Methanol and ethanol significantly ($P < 0.01$) increased DBcAMP-stimulated fluid secretion by 40 ± 16% ($n = 4$) and 57 ± 15% ($n = 5$), respectively. Propanol and butanol significantly ($P < 0.01$) reduced secretion by 34 ± 8% ($n = 5$) and 33 ± 14% ($n = 4$), respectively.

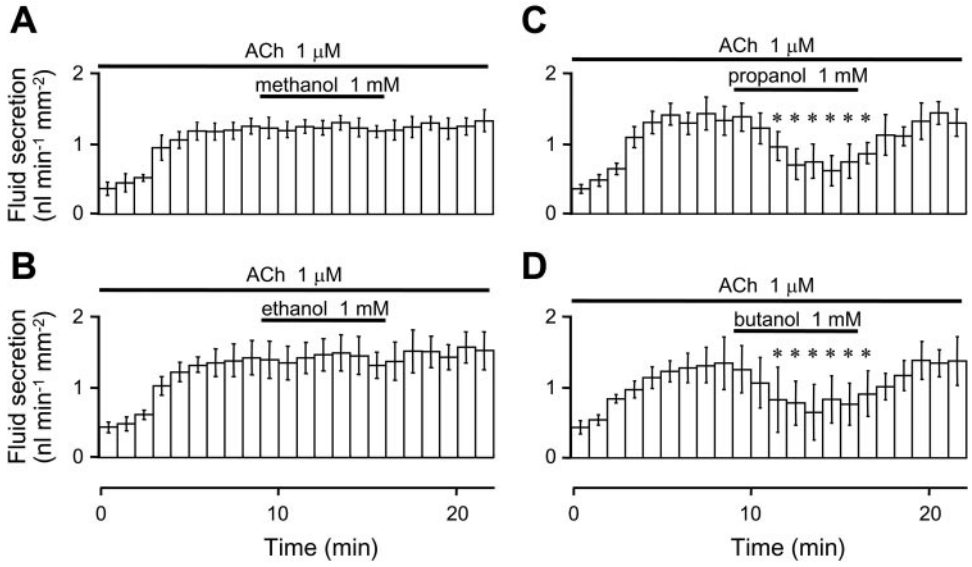

Fig. 3. Effects of *n*-alcohols on fluid secretion (means ± SE) in acetylcholine (ACh)-stimulated ducts. Methanol (*A*; $n = 4$), ethanol (*B*; $n = 5$), propanol (*C*; $n = 5$), or butanol (*D*; $n = 4$) at 1 mM was added to the bath during stimulation with 1 μM ACh. Note that the perfusate was switched from HEPES- to HCO_3^--buffered solution at *time 0*. $*P < 0.05$, significant difference from secretory rate before application of *n*-alcohols.

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ACh-stimulated fluid secretion. ACh (1 μM) increased the fluid secretory rate to 1.29 ± 0.09 $nl \cdot min^{-1} \cdot mm^{-2}$ (n = 18) (Fig. 3). Methanol and ethanol failed to affect ACh-stimulated fluid secretion, but propanol and butanol significantly (P < 0.01) reduced secretion by 46 ± 16% (n = 5) and 48 ± 11% (n = 4), respectively.

Effects of n-Alcohols on $[Ca^{2+}]_i$

In the following studies, we used higher concentrations of secretin (1 nM) and ACh (10 μM) than in the above secretory studies to demonstrate their clear effects on $[Ca^{2+}]_i$ and shorter application periods of alcohols to avoid photobleaching and/or leakage of fura-2. Some of the experiments were repeated under the same protocol used to study fluid secretion (see Fig. 5, *E* and *F*, and Fig. 6, *E* and *F*).

Unstimulated ducts. Methanol and ethanol at 1 mM failed to affect $[Ca^{2+}]_i$, while the application of 1 mM propanol or butanol induced a sustained decrease in $[Ca^{2+}]_i$ (Fig. 4).

Secretin (cAMP)-stimulated ducts. When methanol or ethanol was applied during stimulation with 1 nM secretin, $[Ca^{2+}]_i$ showed a transient increase without a plateau phase (Fig. 5, *A* and *B*). To the contrary, propanol and butanol induced a sustained decrease in $[Ca^{2+}]_i$ that gradually recovered after they were removed from the perfusate phase (Fig. 5, *C* and *D*). In ducts stimulated with 0.3 nM secretin (Fig. 5, *E* and *F*), both the stimulatory (ethanol) and inhibitory (propanol) effects were smaller than those in ducts stimulated with 1 nM secretin. After reaching the nadir, $[Ca^{2+}]_i$ showed a slight tendency to increase during a 7-min application of propanol and returned to control levels after the removal of propanol (Fig. 5*F*). During stimulation with DBcAMP (0.5 mM), methanol and ethanol induced a transient increase in $[Ca^{2+}]_i$, while propanol and butanol induced a sustained decrease (data not shown).

ACh-stimulated ducts. When alcohols were applied during the sustained elevation of $[Ca^{2+}]_i$ induced by ACh (1 or 10 μM), ethanol and methanol failed to affect $[Ca^{2+}]_i$ (Fig. 6, *A*, *B*, and *E*). Propanol and butanol induced a reversible decrease in $[Ca^{2+}]_i$, which showed a tendency to increase before their removal (Fig. 6, *C* and *D*). When propanol was applied during stimulation with 1 μM ACh, $[Ca^{2+}]_i$ exhibited a more stable decrease and returned to the plateau levels after removal of propanol (Fig. 6*F*).

Concentration-Response Relationship

At 0.01 and 0.1 mM, *n*-alcohols failed to affect fluid secretion and $[Ca^{2+}]_i$ in secretin (1 nM)-stimulated ducts (Tables 1 and 2). Both stimulatory (methanol and ethanol) and inhibitory (propanol and butanol) effects on fluid secretion and $[Ca^{2+}]_i$ appeared at 0.3 mM and peaked at 1 mM. Their effects were either reduced or unchanged at 10 mM.

Inhibition of Ca^{2+} Entry Pathway

Under stimulation with secretin, ACh induced a typical peak and plateau response of $[Ca^{2+}]_i$ (Fig. 7*A*). The addition of 1 mM propanol induced a sustained decrease in $[Ca^{2+}]_i$ (Fig. 7*B*). Propanol completely abolished the $[Ca^{2+}]_i$ plateau response to ACh, and only the initial transient response remained. The application of 1 μM thapsigargin, an inhibitor of the Ca^{2+}-ATPase of the endoplasmic reticulum, caused a sustained elevation in $[Ca^{2+}]_i$ (Fig. 7*C*). Propanol inhibited thapsigargin-induced elevation in $[Ca^{2+}]_i$. Under stimulation with secretin, propanol inhibited a transient $[Ca^{2+}]_i$ response to ethanol (Fig. 7*D*); the peak increase in F_{340}-to-F_{380} ratio was significantly (P < 0.05) decreased from the control of 0.096 ± 0.030 (Fig. 2*B*) to 0.029 ± 0.015 and decreased the integrated response by 94%.

Effects of Extracellular Ca^{2+} on Fluid Secretion and $[Ca^{2+}]_i$ in Secretin-Stimulated Ducts

When the superfusate was switched from the standard HCO_3^--buffered solution to the low-Ca^{2+} solution (1 mM $CaCl_2$ + 3 mM EGTA) during stimulation with secretin (1 nM), the fluid secretory rate reversibly decreased by 45 ± 11% (n = 5; P < 0.05) (Fig. 8*A*). When the superfusate was switched to the Ca^{2+}-free solution (0 $CaCl_2$ + 5 mM EGTA), fluid secretion was almost abolished (n = 4; P < 0.01) (Fig. 8*B*). The inhibition was partially reversible. In the same experimental conditions, $[Ca^{2+}]_i$ decreased by 0.068 ± 0.028 (Δ ratio) with low Ca^{2+} in the bath (Fig. 8*C*) and decreased by 0.090 ± 0.024 without Ca^{2+} in the bath (Fig. 8*D*), respectively (n = 4).

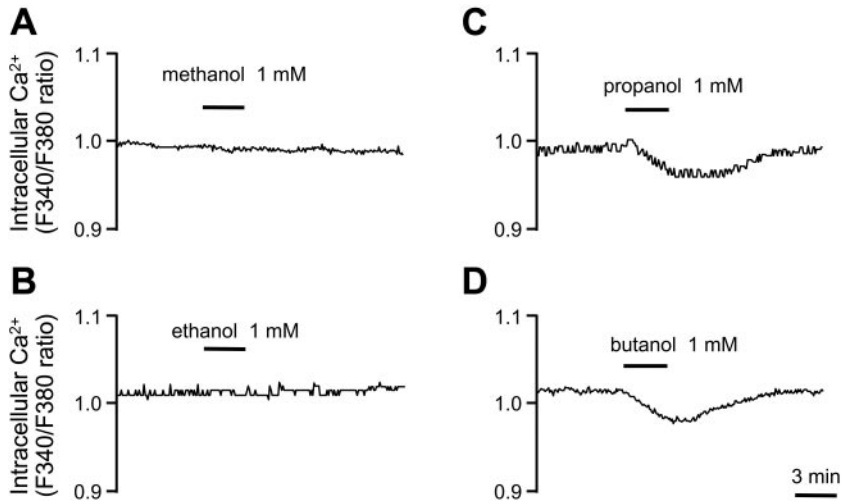

Fig. 4. Effects of *n*-alcohols on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in unstimulated ducts. The closed ducts were superfused with the standard HCO_3^--CO_2-buffered solution. Methanol (*A*), ethanol (*B*), propanol (*C*), or butanol (*D*) at 1 mM was added to the bath as indicated. Changes in fura-2 fluorescence (F_{340} to F_{380}) ratio indicate changes in $[Ca^{2+}]_i$ in pancreatic duct cells. Each trace is representative of four experiments.

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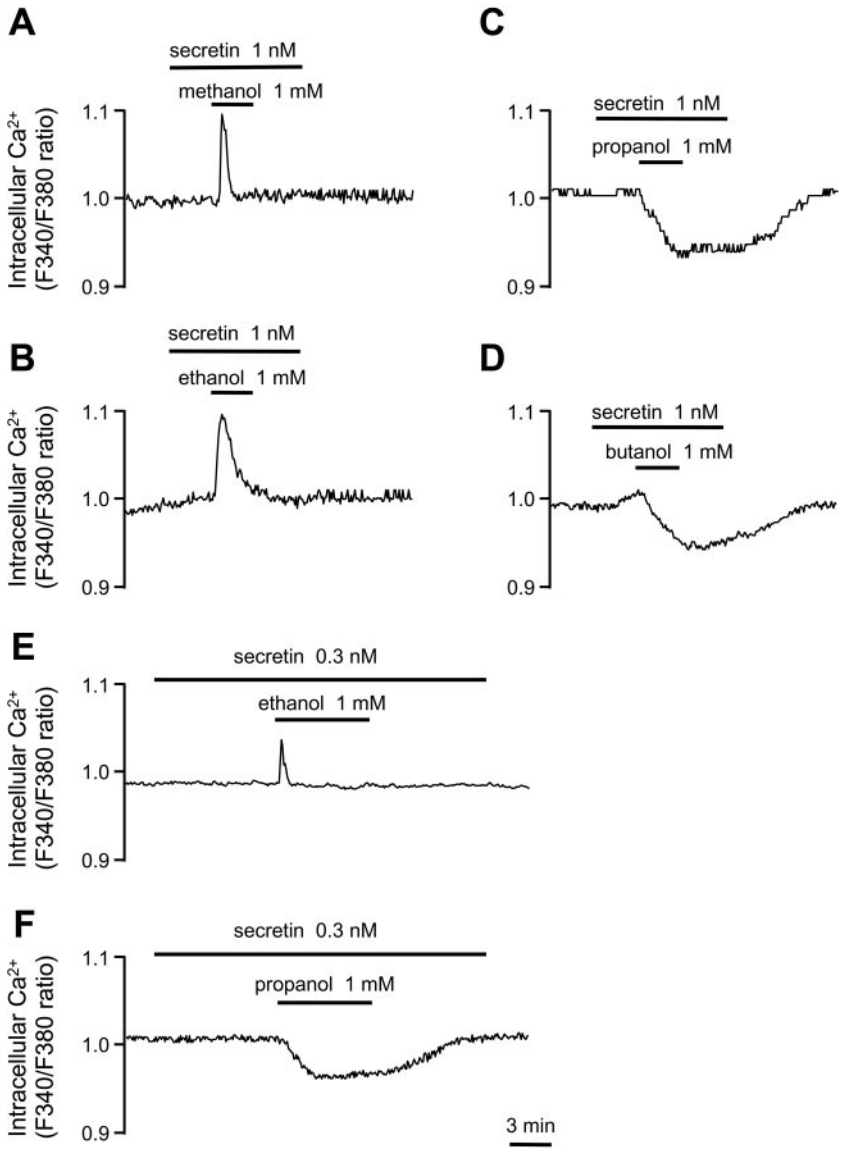

Fig. 5. Effects of *n*-alcohols on $[Ca^{2+}]_i$ in secretin-stimulated ducts. Methanol (*A*), ethanol (*B* and *E*), propanol (*C* and *F*), or butanol (*D*) at 1 mM was added to the bath during stimulation with 1 nM (*A–D*) or 0.3 nM (*E* and *F*) of secretin. Each trace is representative of four experiments.

DISCUSSION

Dual Actions of n-Alcohols on Pancreatic Duct Cells

In this study, we tested a series of short-chain *n*-alcohols and found a distinct cutoff point between ethanol (C2) and propanol (C3) in their effects on fluid secretion and $[Ca^{2+}]_i$ in guinea pig pancreatic duct cells. This cutoff point for *n*-alcohols is the shortest that has been reported (20). The actions of methanol on duct cell functions were very similar to those of ethanol (31); both augmented cAMP-mediated fluid secretion and induced a transient increase of $[Ca^{2+}]_i$ but failed to affect spontaneous or ACh-stimulated secretion and $[Ca^{2+}]_i$. Unexpectedly, propanol (C3) and butanol (C4) showed the opposite effects; both reduced fluid secretion as well as $[Ca^{2+}]_i$, regardless of the stimuli used. Both stimulatory and inhibitory effects of *n*-alcohols were rapid in onset time and disappeared quickly and completely upon removal from the perfusate. The effective concentration ranges (0.3–10 mM) were also very similar among alcohols (Tables 1 and 2). Because appreciable changes in the fluidity of cell membranes occur at 11–44 mM ethanol (4), the observed effects in this study probably were not induced by perturbation of membrane lipids. In addition, the membrane disordering potency of *n*-alcohol increases logarithmically with the number of methylene groups. Hence the disordering potency of propanol is 3.6 times that of ethanol, and the disordering potency of butanol is 11.9 times that of ethanol (23). If propanol and butanol act via membrane disordering, their effective concentration ranges should be different, i.e., lower for butanol than for methanol, which we never observed. Therefore, it is very likely that they act directly on proteins, but at different sites.

Transient Cessation of Spontaneous Fluid Secretion

The transient cessation of spontaneous fluid secretion by methanol and ethanol (Fig. 1) is most likely due to their osmotic effects until they reach equilibrium with the luminal

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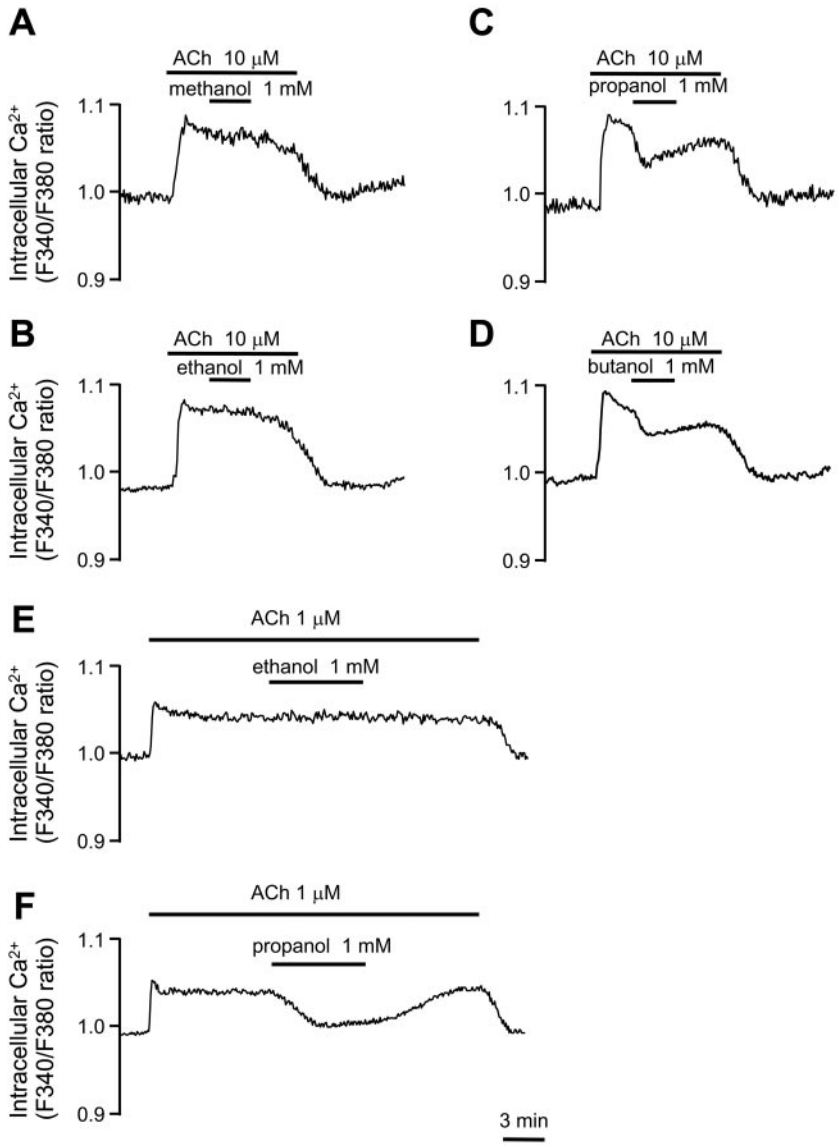

Fig. 6. Effects of *n*-alcohols on $[Ca^{2+}]_i$ in ACh-stimulated ducts. Methanol (*A*), ethanol (*B* and *E*), propanol (*C* and *F*), or butanol (*D*) at 1 mM was added to the bath during stimulation with 10 μM ACh (*A–D*) or 1 μM ACh (*E* and *F*). Each trace is representative of four experiments.

fluid (31). As low as 1 mosmol/l gradient between the lumen and the perfusate was sufficient to completely stop spontaneous fluid secretion from the ducts. The absence of such effects of propanol and butanol is probably because their diffusion rates through the cell membranes are much faster than those of methanol and ethanol; the membrane-buffer partition coefficients are 0.036 for methanol, 0.096 for ethanol, 0.438 for propanol, and 1.52 for butanol (23).

Table 1. *Relationship of n-alcohol concentrations and responses in fluid secretion stimulated with 0.3 nM secretin in isolated pancreatic ducts*

	n-Alcohol				
	0.01 mM	0.1 mM	0.3 mM	1 mM	10 mM
Methanol	0.3±4.0	1.2±5.0	31.0±9.6*	40.9±6.9*	28.4±9.4*
Ethanol	0.5±3.2	1.9±6.0	49.7±9.6*	85.2±6.3*	40.2±11.4*
Propanol	−2.8±5.9	−3.6±6.2	−19.8±8.2	−44.3±12.7*	−50.0±8.9*
Butanol	−2.4±5.9	−3.5±6.0	−18.8±8.3	−44.0±9.0*	−49.2±8.9*

Values are means ± SE; $n = 4$ experiments. Data are %increase from secretory rate without alcohol. *$P < 0.05$, significant difference from control (0 mM).

Augmentation by Methanol and Ethanol

Because the augmentation of fluid secretion and the $[Ca^{2+}]_i$ increase by methanol and ethanol were observed only under cAMP stimulation, both alcohols should act at a point distal to the production of cAMP. Transient elevation of $[Ca^{2+}]_i$ by ethanol is probably caused by the activation of plasma membrane Ca^{2+} channels, because the $[Ca^{2+}]_i$ increase was abolished by the removal of extracellular Ca^{2+} but not by the depletion of intracellular Ca^{2+} stores with thapsigargin (31). The activation of Ca^{2+} influx is necessary for augmentation because it was completely abolished when changes in $[Ca^{2+}]_i$ were buffered with BAPTA (31). Methanol acted on ductal fluid secretion and $[Ca^{2+}]_i$ in the same manner (Figs. 1–6), as well as the same concentration range (Tables 1 and 2), as

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Table 2. *Relationship of n-alcohol concentrations and responses in $[Ca^{2+}]_i$ stimulated with 1 nM secretin in isolated pancreatic ducts*

	n-Alcohol				
	0.01 mM	0.1 mM	0.3 mM	1 mM	10 mM
Methanol	0.001±0.004	0.003±0.003	0.052±0.015	0.122±0.038*	0.041±0.022
Ethanol	0.002±0.003	0.003±0.002	0.045±0.011*	0.096±0.030*	0.038±0.009*
Propanol	−0.003±0.006	−0.008±0.009	−0.041±0.011*	−0.082±0.022*	−0.088±0.019*
Butanol	−0.003±0.008	−0.007±0.010	−0.039±0.023	−0.064±0.020*	−0.078±0.025*

Values are means ± SE; $n = 4$ experiments. Data are Δratio compared with data without alcohol. *$P < 0.05$, significant difference from control (0 mM).

ethanol. Because they differed only in their potency (methanol < ethanol), they probably acted on the same sites. Possible sites for their actions are a regulatory site for cAMP/protein kinase A of the plasma membrane Ca^{2+} channel and a cAMP-regulated anion channel and/or transporter such as CFTR on the luminal membrane and Na^+-HCO_3^- cotransport on the basolateral membrane.

Inhibition by Propanol and Butanol

The inhibition of ductal fluid secretion and the reduction of $[Ca^{2+}]_i$ caused by propanol and butanol under resting conditions were also observed after stimulation with secretin, cAMP, or ACh (Figs. 1–6). The $[Ca^{2+}]_i$ decrease started immediately after the application of propanol or butanol and reached the nadir within ~3 min. The secretory rate started to decrease after a delay of 2 min but reached the nadir within 2–3 min. Both $[Ca^{2+}]_i$ and secretion returned to preapplication control levels within ~5 min. The minimal effective concentration of propanol and butanol was 0.3 mM for both fluid secretion and $[Ca^{2+}]_i$, and no further reductions were observed at 1–10 mM (Tables 1 and 2). Furthermore, the magnitudes of inhibition were similar with all stimulants tested. These data strongly suggest that reductions of $[Ca^{2+}]_i$ induced by propanol and butanol are closely related to their inhibitory effects on ductal fluid secretion.

Propanol and Butanol Inhibit Ca^{2+} Influx

As in acinar cells (30), ACh induced a biphasic elevation of $[Ca^{2+}]_i$ in pancreatic duct cells; the initial peak by Ca^{2+} release from intracellular stores was followed by a plateau phase caused by influx of extracellular Ca^{2+} via plasma membrane Ca^{2+} channels (2). The application of propanol or butanol caused a reversible inhibition of the plateau phase (Fig. 6). The prior application of propanol completely abolished the plateau phase Ca^{2+} response to ACh, but not the initial peak response (Fig. 7*B*), indicating that propanol inhibits Ca^{2+} influx via plasma membrane Ca^{2+} channels. Furthermore, propanol inhibited the elevation of $[Ca^{2+}]_i$ due to the increased Ca^{2+} influx by thapsigargin (Fig. 7*C*) as well as ethanol-induced Ca^{2+} entry (Fig. 7*D*). These inhibitory effects of propanol and butanol are very similar to those of lanthanum, an inhibitor of the plasma membrane Ca^{2+} channels (12). The molecule responsible for Ca^{2+} influx in duct cells, however, has not been identified yet (7), and the exact sites of action of propanol and butanol remain to be studied.

Ca^{2+} Influx Is Necessary for Ductal Fluid Secretion

ACh evokes fluid secretion via elevation of $[Ca^{2+}]_i$ in isolated pancreatic ducts (2); hence propanol and butanol probably inhibited ACh-stimulated fluid secretion by their

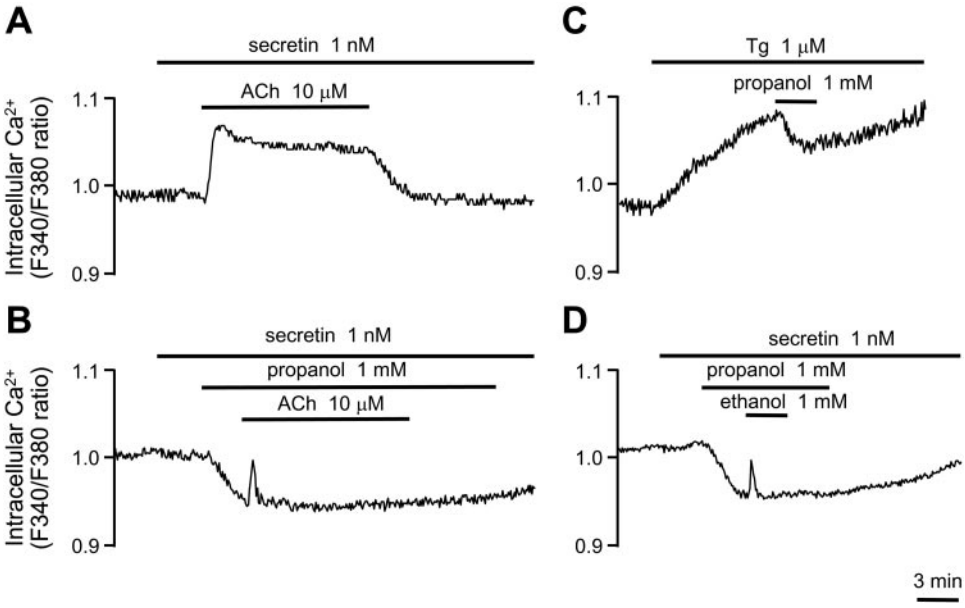

Fig. 7. The inhibition of $[Ca^{2+}]_i$ by propanol. *A* and *B*: $[Ca^{2+}]_i$ in pancreatic duct cells stimulated with 1 nM secretin plus 10 μM ACh with (*B*) or without (*A*) 1 mM propanol. *C* and *D*: effect of 1 mM propanol on $[Ca^{2+}]_i$ in duct cells in response to 1 μM thapsigargin (*C*) and 1 nM secretin plus 1 mM ethanol (*D*). Each trace is representative of four experiments.

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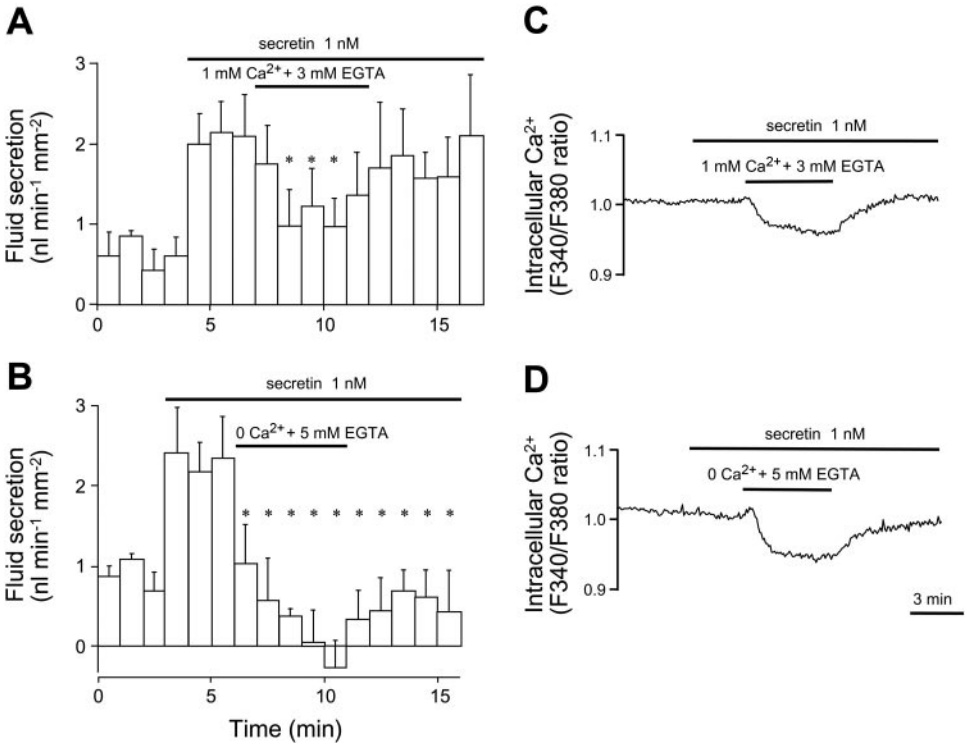

Fig. 8. Effects of extracellular Ca^{2+} on secretin-stimulated fluid secretion and $[Ca^{2+}]_i$. *A* and *C*: bath perfusate was switched from the standard HCO_3^--buffered solution to the low-Ca^{2+} solution [1 mM $CaCl_2$ + 3 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA)] during stimulation with 1 nM secretin. *B* and *D*: bath perfusate was switched from the standard HCO_3^--buffered solution to the Ca^{2+}-free solution (0 $CaCl_2$ + 5 mM EGTA). Means ± SE of four (*A*) and five (*B*) experiments. $*P < 0.05$, significant difference from secretory rate before removal of Ca^{2+} in the bath. *C* and *D*: each trace is representative of four experiments with $[Ca^{2+}]_i$.

inhibitory effects on the Ca^{2+} influx pathway. The inhibition of secretin- and cAMP-stimulated fluid secretion by these alcohols is rather surprising because cAMP and Ca^{2+} pathways have been considered to be independent of each other in pancreatic duct cells (3). A simultaneous application of secretin and ACh elicits an additive effect of each stimulant alone in guinea pigs (31) but an inhibitory interaction in rats (8). In agreement with previous studies (26), removal of extracellular Ca^{2+} quickly reduced $[Ca^{2+}]_i$ (Fig. 8, *C* and *D*). Removal of extracellular Ca^{2+} reduced secretin-stimulated fluid secretion by 26% in rat pancreatic ducts (1). In guinea pigs, however, fluid secretion completely ceased in the absence of extracellular Ca^{2+} (Fig. 8*B*), which indicates that continuous Ca^{2+} influx or a certain level of $[Ca^{2+}]_i$ is necessary to maintain secretin- or cAMP-stimulated fluid secretion. Although we are unable to exclude the possibility that removal of extracellular Ca^{2+} affects the permeability of tight junction and consequently inhibits fluid secretion, similarities in the fluid and $[Ca^{2+}]_i$ responses to propanol and buthanol and to the removal of extracellular Ca^{2+} strongly suggest that their inhibitory effects on the ductal fluid responses to secretin are attributable to their inhibitory action on Ca^{2+} influx.

Hypothetical Mechanism for Dual Actions of n-Alcohols

Ligand-gated ion channels and voltage-gated Ca^{2+} channels have been found to be sensitive to alcohols (5, 22). Alcohols exhibit a cutoff effect that is dependent on their alkyl chain length (20), which varies widely (4–13 carbons) among the ion channels. In the present study, the cutoff point was between two and three carbons. The most attractive hypothesis to account for the different cutoff points of alcohols is that their target sites are amphiphilic pockets in the protein structure (9, 10, 29). The binding of alcohols to preformed pockets, one for methanol and ethanol and the other for propanol and butanol, might cause slight rearrangements of certain amino acids that affect the gating of Ca^{2+} channels; the former induces the opening, and the latter induces the closure.

In summary, we have shown that *n*-alcohols have dual effects on pancreatic ductal fluid secretion, depending on the length of their alkyl chain; methanol and ethanol cause the augmentation, while propanol and butanol cause inhibition. Both stimulatory and inhibitory effects are probably caused by their direct actions on ion channel or transporter proteins rather than by their indirect actions on membrane fluidity. Although the exact molecule remains to be identified, the inhibition of membrane Ca^{2+} channels by propanol and butanol unveils the importance of Ca^{2+} influx in cAMP-mediated fluid secretion. The diverse effects of aliphatic alcohols have been attributed to their CNS-depressant actions or to toxic effects of their metabolites. Our study suggests that these alcohols may also exert their actions on any cell by interacting with preformed pockets on a variety of ion channel or transporter proteins.

ACKNOWLEDGMENTS

We thank Dr. V. Wray for advice.

Present address of M. Kitagawa: National Center for Geriatrics and Gerontology, Ohbu, Japan.

Present address of T. Hayakawa: Meijo Hospital, Nagoya, Japan.

GRANTS

This study was supported by grants from the Japan Society for the Promotion of Science, the Ministry of Education, Science and Technology, Japan, and the Ministry of Health, Labor and Welfare, Japan (to S. Naruse).

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REFERENCES

1. **Ashton N, Argent BE, and Green R.** Characteristics of fluid secretion from isolated rat pancreatic ducts stimulated with secretin and bombesin. *J Physiol* 435: 533–546, 1991.
2. **Ashton N, Evans RL, Elliott AC, Green R, and Argent BE.** Regulation of fluid secretion and intracellular messengers in isolated rat pancreatic ducts by acetylcholine. *J Physiol* 471: 549–562, 1993.
3. **Case RM and Argent BE.** Pancreatic duct cell secretion: control and mechanisms of transport. In: *The Pancreas: Biology, Pathobiology and Disease* (2nd ed.), edited by Go VLW, DiMagno EP, Gardner JD, Labenthal E, Reher HA, and Scheele GA. New York: Raven, 1993, p. 301–350.
4. **Chin JH and Goldstein DB.** Electron paramagnetic resonance studies of ethanol on membrane fluidity. *Adv Exp Med Biol* 85A: 111–122, 1977.
5. **Crews FT, Morrow AL, Criswell H, and Breese G.** Effects of ethanol on ion channels. *Int Rev Neurobiol* 39: 283–367, 1996.
6. **Criddle DN, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R.** Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. *Proc Natl Acad Sci USA* 101: 10738–10743, 2004.
7. **Elliott AC.** Recent developments in non-excitable cell calcium entry. *Cell Calcium* 30: 73–93, 2001.
8. **Evans RL, Ashton N, Elliott AC, Green R, and Argent BE.** Interactions between secretin and acetylcholine in the regulation of fluid secretion by isolated rat pancreatic ducts. *J Physiol* 496: 265–273, 1996.
9. **Franks NP, Jenkins A, Conti E, Lieb WR, and Brick P.** Structural basis for the inhibition of firefly luciferase by a general anesthetic. *Biophys J* 75: 2205–2211, 1998.
10. **Franks NP and Lieb WR.** Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects. *Nature* 316: 349–351, 1985.
11. **Furuya S, Naruse S, Ko SBH, Ishiguro H, Yoshikawa T, and Hayakawa T.** Distribution of aquaporin 1 in the rat pancreatic duct system examined with light and electron microscopic immunohistochemistry. *Cell Tissue Res* 308: 75–86, 2002.
12. **Hug MJ, Pahl C, and Novak I.** Calcium influx pathways in rat pancreatic ducts. *Pflügers Arch* 432: 278–285, 1996.
13. **Ishiguro H, Naruse S, Steward MC, Kitagawa M, Ko SB, Hayakawa T, Case RM.** Fluid secretion in interlobular ducts isolated from guinea-pig pancreas. *J Physiol* 511: 407–422, 1998.
14. **Ishiguro H, Steward MC, Lindsay ARG, and Case RM.** Accumulation of intracellular HCO_3^- by Na^+-HCO_3^- cotransport in interlobular ducts from guinea-pig pancreas. *J Physiol* 495: 169–178, 1996.
15. **Ishiguro H, Steward MC, Sohma Y, Kubota T, Kitagawa M, Kondo T, Case RM, Hayakawa T, and Naruse S.** Membrane potential and bicarbonate secretion in isolated interlobular ducts from guinea-pig pancreas. *J Gen Physiol* 120: 617–628, 2002.
16. **Jones AW and Holmgren P.** Comparison of blood-ethanol concentration in deaths attributed to acute alcohol poisoning and chronic alcoholism. *J Forensic Sci* 48: 874–879, 2003.
17. **Kao JPY.** Practical aspects of measuring [Ca^{2+}] with fluorescent indicators. In: *A Practical Guide to the Study of Calcium in Living Cells*. San Diego, CA: Academic Press, 1994, p. 155–181.
18. **Ko SBH, Naruse S, Kitagawa M, Ishiguro H, Furuya S, Mizuno N, Wang Y, Yoshikawa T, Suzuki A, Shimano S, and Hayakawa T.** Aquaporins in rat pancreatic interlobular ducts. *Am J Physiol Gastrointest Liver Physiol* 282: G324–G331, 2002.
19. **Ko SBH, Naruse S, Kitagawa M, Ishiguro H, Murakami M, and Hayakawa T.** Arginine vasopressin inhibits fluid secretion in guinea pig pancreatic duct cells. *Am J Physiol Gastrointest Liver Physiol* 277: G48–G54, 1999.
20. **Korpi ER, Mäkelä R, and Uusi-Oukari M.** Ethanol: novel actions on nerve cell physiology explain impaired functions. *News Physiol Sci* 13: 164–170, 1998.
21. **Laurence DR and Bennett PN.** *Clinical Pharmacology* (6th ed.). Edinburgh, UK: Churchill Livingstone, 1987.
22. **Little HJ.** The contribution of electrophysiology to knowledge of the acute and chronic effects of ethanol. *Pharmacol Ther* 84: 333–353, 1999.
23. **Lyon RC, McComb JA, Schreurs J, and Goldstein DB.** A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. *J Pharmacol Exp Ther* 218: 669–675, 1981.
24. **Naruse S, Kitagawa M, and Ishiguro H.** Molecular understanding of chronic pancreatitis: a perspective on the future. *Mol Med Today* 5: 493–499, 1999.
25. **Peoples RW, Li C, and Weight FF.** Lipid vs. protein theories of alcohol action in the nervous system. *Annu Rev Pharmacol Toxicol* 36: 185–201, 1996.
26. **Stuenkel EL and Hootman SR.** Secretagogue effects on intracellular calcium in pancreatic duct cells. *Pflügers Arch* 416: 652–658, 1990.
27. **Suzuki A, Naruse S, Kitagawa M, Ishiguro H, Yoshikawa T, Ko SBH, Yamamoto A, Hamada H, and Hayakawa T.** 5-Hydroxytryptamine strongly inhibits fluid secretion in guinea-pig pancreatic duct cells. *J Clin Invest* 108: 749–756, 2001.
28. **Werner J, Saghir M, Warshaw AL, Lewandrowski KB, Laposata M, Iozzo RV, Carter EA, Schatz RJ, and Fernandez-Del Castillo C.** Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol. *Am J Physiol Gastrointest Liver Physiol* 283: G65–G73, 2002.
29. **Wick MJ, Mihic SJ, Ueno S, Mascia MP, Trudell JR, Brozowski SJ, Ye Q, Harrison NL, and Harris RA.** Mutations of γ-aminobutyric acid and glycine receptors change alcohol cutoff: evidence for an alcohol receptor? *Proc Natl Acad Sci USA* 95: 6504–6509, 1998.
30. **Williams JA and Yule DI.** Stimulus-secretion coupling in pancreatic acinar cells. In: *The Pancreas: Biology, Pathobiology and Disease* (2nd ed.), edited by Go VLW, DiMagno EP, Gardner JD, Labenthal E, Reher HA, and Scheele GA. New York, Raven, 1993, p. 167–189.
31. **Yamamoto A, Ishiguro H, Ko SBH, Suzuki A, Wang Y, Hamada H, Mizuno N, Kitagawa M, Hayakawa T, and Naruse S.** Ethanol induces fluid hypersecretion in guinea-pig pancreatic duct cells. *J Physiol* 551: 917–926, 2003.

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