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Adrenoceptor-mediated secretion across the rat colonic epithelium

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Abstract

Norepinephrine evoked a biphasic change in short-circuit current (Isc) across the proximal and distal colon of the rat. The (1) phase of the current response consisted of a transient increase, which was followed by a long-lasting decrease during the (2) phase. The (1) phase, which is assumed to represent Cl^- secretion, was resistant against classical adrenoceptor antagonists, but was inhibited by the β_3-adrenoceptor antagonist 3-(2-ethylpenoxy)-1-[(1*S*-1,2,3,4-tetrahydronaphth-1-ylaminol-(2*S*)-propranol oxalate (SR 59230A) in the proximal colon and by the non-selective β-adrenoceptor antagonist bupranolol in both colonic segments. Vice versa, the increase in Isc was mimicked by the β_3-adrenoceptor agonist, (R^*,R^*)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid (BRL 37344). The (2) phase of the norepinephrine-induced Isc, which is assumed to represent K^+ secretion, was inhibited by yohimbine in the proximal colon, suggesting the mediation by α_2-adrenoceptors, whereas in the distal colon, both α- and β-adrenoceptors are involved, as shown by the sensitivity against, e.g. phentolamine and propranolol. These adrenoceptors seem to be located — at least in part — at extraepithelial sites because the (1) phase of the norepinephrine response was sensitive to indomethacin, and the (2) phase, both to indomethacin and tetrodotoxin.

Keywords: Adrenoceptor; β_3-Adrenoceptor; Cl^- channel; K^+ channel; Ionic secretion; Colon, rat

1. Introduction

Electrolyte transport in the gut is under the control of neurotransmitters, hormones and paracrine substances (for review, see e.g. Cook and Reedix, 1994). Recently, we studied the effect of epinephrine, which evokes a biphasic secretory response leading to a transient increase in short-circuit current (Isc), followed by a long lasting decrease (Hörger et al., 1998). The initial Isc response is evoked by a Cl^- secretion, as shown by anion substitution experiments and by the sensitivity against bumetanide, an inhibitor of the Na^+–K^+–$2Cl^-$ cotransporter. This Cl^- secretion exhibited a great variability between tissues from different animals, as it was observed — depending on the colonic segment — only in about 70–80% of the experiments. In contrast, the (2) phase of the epinephrine response, i.e. the decrease in Isc, was consistently found in nearly all tissues investigated. The late catecholamine response is caused by a sustained K^+ secretion, as revealed by measurements of unidirectional ion fluxes.

Surprisingly, the (1) phase of the catecholamine-induced Isc proved to be resistant against all tested α- (phentolamine, prazosin, yohimbine) and β-adrenoceptors (propranolol, atenolol, (±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI-118551)) antagonists (Hörger et al., 1998). Therefore, one aim of the present study was to find out, which kind of adrenoceptor may be involved in the induction of Cl^- secretion by catecholamines. In addition, the concentration of epinephrine ($5 \cdot 10^{-6}$ mol l^{-1}), which is necessary to induce a maximal Cl^- or K^+ secretion, is probably never observed in vivo, when the hormone is secreted from the adrenal gland, reaching the gut via the circulation. However, norepinephrine, which is released from sympathetic nerve endings within the intestinal wall, may reach much higher local concentrations. Therefore, in the present study, the secretory effect of norepinephrine on the colonic mucosa was investigated.

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2. Materials and methods

2.1. Solutions

The Ussing chamber experiments were carried out in a bathing solution containing (mmol l^{-1}): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with carbogen (5% CO_2/95% O_2) and kept at a temperature of 37°C; pH was 7.4. For the Cl^--free solution, NaCl was substituted by Na gluconate.

2.2. Tissue preparation

Wistar rats were used with a weight of 180–220 g. The animals had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away by hand to obtain the mucosa–submucosa preparation of the colon. The appearance of palm leaf-like striae was taken as criterion to define the border between the distal and the proximal colon (Lindström et al., 1979).

2.3. Short-circuit current measurement and data evaluation

The tissue was fixed in a modified Ussing chamber, bathed with a volume of 3.5 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen) with correction for solution resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (Isc) was continuously recorded and tissue conductance was measured every minute.

In general, the response to norepinephrine was first tested under control conditions, i.e. in the absence of any inhibitors. Then, the serosal compartment was washed three times with 5 × the chamber volume, before a putative inhibitor was administered. 10–15 min later, norepinephrine was administered a second time and the current response, evoked by the catecholamine in the presence of the blocker, was compared with that in the absence of the putative inhibitor. Control experiments revealed that there were no significant differences in the Isc responses, when norepinephrine was administered three times to the same tissue ($n = 8$ both for the distal and the proximal colon).

The presence of a (1) phase of the norepinephrine response was defined as an increase in Isc of at least 0.1 μEq h^{-1} cm^{-2} within the first 2 min after administration of the catecholamine. When the amplitude of the (1) phase was compared in one tissue in the absence and presence of putative inhibitors, only tissues were included in the statistics, which exhibited an increase in Isc under control conditions, i.e. in the absence of the putative inhibitor. In the tables, the number of tissues, in which a (1) phase was found, is given in parenthesis in relation to the total number of tissues tested. All data in the tables are given as difference (ΔIsc) to the baseline Isc just prior to the administration of the respective drug.

2.4. Drugs

3-(2-ethylpenoxy)-1-[(1*S*-1,2,3,4-tetrahydronaphth-1-ylaminol-(2*S*)-propranol oxalate (SR 59230 A, gift from Sanofi Winthrop, Milano, Italy) and phentolamine methanesulfate (Aldrich, Steinheim, Germany) were dissolved in dimethylsulfoxide (DMSO, final concentration 3 μl ml^{-1}). Indomethacin and yohimbine hydrochloride were dissolved in ethanol (final maximal concentration 1 μl ml^{-1}). Atenolol (gift from Zeneca, Plankstadt, Germany), bupranolol (gift from Schwarz Pharma, Monheim, Germany), (R^*,R^*)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino] propyl]phenoxyacetic acid sodium salt (BRL 37344), 4-[3-[(1,1-dimethylethyl)amino]2-hydroxypropoxy]1,3 dihydro-2H-benzimidazol-2one hydrochloride (CGP 12177), (±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI-118551, all three drugs from Tocris, Bristol, UK), norepinephrine bitartrate, prazosin hydrochloride (gift from Pfizer, Karlsruhe, Germany) and propranolol (Aldrich) were dissolved in aqueous stock solutions diluted in salt buffer just before use. Tetrodotoxin was dissolved as a stock solution in citrate buffer (20 mmol l^{-1}). If not indicated differently, drugs were from Sigma, Deisenhofen, Germany.

2.5. Statistics

Results are given as means ± one standard error of the mean (S.E.M.). The significance of differences was tested by paired or unpaired two-tailed Student's *t*-test or a *U*-test. An *F*-test was applied to decide which test method was to be used. If more data sets had to be compared, a one-way analysis of variances was applied.

3. Results

3.1. Baseline effects of norepinephrine

After an equilibration time of 60 min, the baseline short-circuit current (Isc) amounted to 3.0 ± 0.6 μEq h^{-1} cm^{-2} ($n = 6$) in the proximal and 2.6 ± 0.1 μEq h^{-1} cm^{-2} ($n = 6$) in the distal colon. Norepinephrine concentration-dependently induced a change in Isc in a biphasic manner in both colonic segments, i.e. a first transient increase, which was followed by a long-lasting decrease (Fig. 1). A maximal effect was observed at a concentration

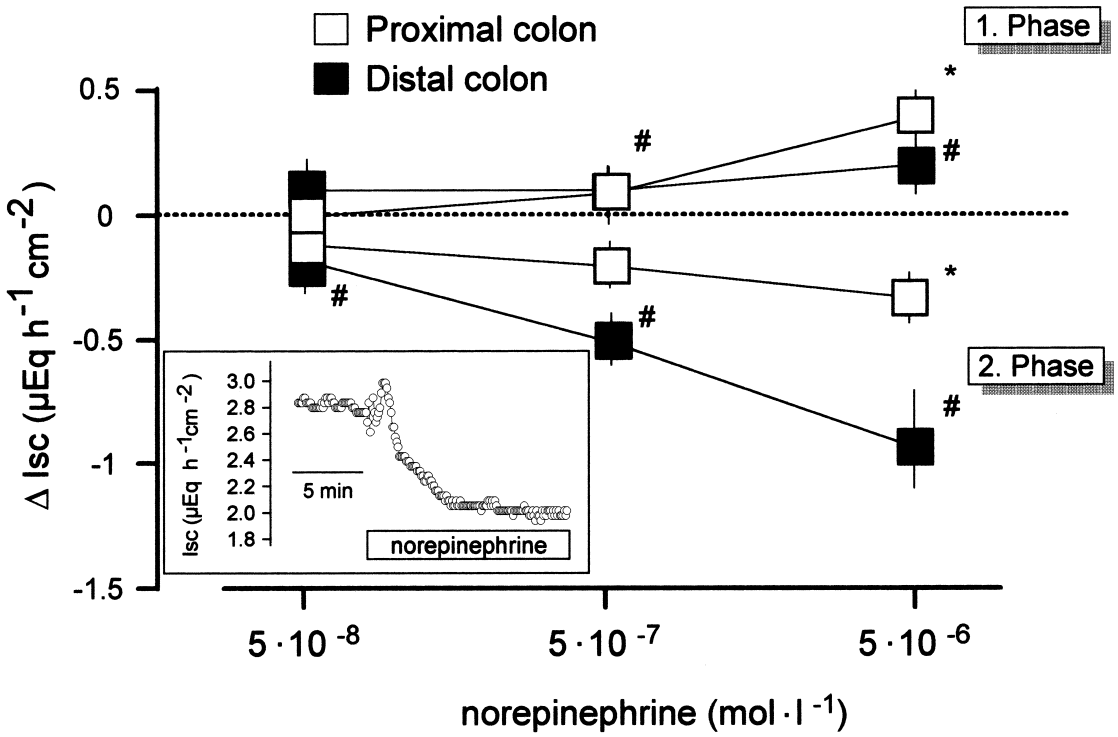

Fig. 1. Concentration-dependent effect of norepinephrine on Isc in the proximal (open squares) and distal colon (filled squares). The current response during the 1. phase (increase in Isc) and the 2. phase (decrease in Isc) of the norepinephrine-response is given. The inlet depicts a typical time course of the change in Isc induced by norepinephrine ($5 \cdot 10^{-6}$ mol $\cdot$ l^{-1} at the serosal side). Data are given as ΔIsc, i.e. difference to the baseline just prior administration of the catecholamine. $^{*}P < 0.05$ versus baseline prior administration of norepinephrine in the proximal colon, $\#P < 0.05$ versus baseline prior administration of norepinephrine in the distal colon. Values are means (symbols) ± S.E.M. (error bars), $n = 6$.

of $5 \cdot 10^{-6}$ mol l^{-1}, which was therefore used for all further experiments.

The two phases of the norepinephrine response differed strongly in the constancy, with which they were found. An initial increase in Isc was only observed in 60 out of 98 tissues (= 61%) in the proximal and in 49 out of 103 tissues (= 48%) in the distal colon. In contrast, nearly all tissues (97/98 in the proximal and 100/103 tissues in the distal colon) exhibited the (2) phase, characterized by the long-lasting decrease in Isc. The tissues did not desensitize when norepinephrine ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side) was administered three times to the same preparation ($n = 8$ for each colonic segment; data not shown). Therefore, in all subsequent experiments, the response to norepinephrine was first tested in the absence of any drugs and then in the presence of putative inhibitors.

In the case of epinephrine, a part of the secretory response was mediated by enteric neurons (Hörger et al., 1998). Therefore, the effect of norepinephrine was measured in the presence of the neuronal toxin, tetrodotoxin (10^{-6} mol l^{-1} at the serosal side). In both colonic segments, the neurotoxin itself induced a decrease in Isc (see Table 1 for the effect of the inhibitors themselves on Isc). In the presence of tetrodotoxin, the (1) phase of the norepinephrine effect was not inhibited. In contrast, in the presence of the neuronal blocker the norepinephrine-induced increase in Isc was observed in every tissue tested in both colonic segments, indicating an inhibitory effect of enteric neurons on the catecholamine-induced Cl^{-} secretion, which is abolished by tetrodotoxin. However, the (2) phase of the norepinephrine response was reduced by 50% by the neurotoxin in the proximal colon (0.8 ± 0.2 μEq

Table 1
Effect of different inhibitors on Isc

Drugs	Proximal colon	Distal colon	n
	Δ Isc (μEq · h^{-1} · cm^{-2})		
Tetrodotoxin	−0.3 ± 0.2	−0.6 ± 0.1[a]	10
Indomethacin	−1.1 ± 0.2[a]	−0.9 ± 0.1[a]	9–10
Phentolamine	−0.2 ± 0.1	−0.7 ± 0.2[a]	10–11
Prazosin	0.0 ± 0.1	0.2 ± 0.1[a]	9
Yohimbine	−0.1 ± 0.1	−0.8 ± 0.1[a]	6–8
Propranolol	−0.1 ± 0.1	−0.6 ± 0.1[a]	7–8
Atenolol	−0.1 ± 0.1	0.2 ± 0.1[a]	9–10
ICI-118551	−0.1 ± 0.1	−0.5 ± 0.1[a]	9–10

Atenolol (10^{-4} mol l^{-1} at the serosal side), ICI-118551 (10^{-5} mol l^{-1} at the serosal side), indomethacin (10^{-6} mol l^{-1} at the serosal side), phentolamine (10^{-4} mol l^{-1} at the serosal side), prazosin (10^{-6} mol l^{-1} at the serosal side), propranolol ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side), tetrodotoxin (10^{-6} mol l^{-1} at the serosal side), yohimbine (10^{-5} mol l^{-1} at the serosal side), Values are means ± S.E.M.

[a] $P < 0.05$ vs. baseline just prior administration of the respective drug.

Table 2
Effect of tetrodotoxin and indomethacin on the norepinephrine-induced Isc

	Proximal colon		Distal colon		*n*
	ΔIsc (μEq h^{-1} cm^{-2})				
	(1) Phase	(2) Phase	(1) Phase	(2) Phase	
Norepinephrine	0.2 ± 0.0^{a} (5/10)	-0.8 ± 0.2^{a}	0.3 ± 0.1^{a} (5/10)	-1.0 ± 0.1^{a}	10
+ tetrodotoxin	0.2 ± 0.1	$-0.4 \pm 0.1^{a,b}$	0.3 ± 0.1^{a}	-0.1 ± 0.1^{b}	
Norepinephrine	0.2 ± 0.0^{a} (6/9)	-0.7 ± 0.1^{a}	0.2 ± 0.0^{a} (3/10)	-0.8 ± 0.1^{a}	9–10
+ indomethacin	0.1 ± 0.0^{b}	$-0.1 \pm 0.0^{a,b}$	0.0 ± 0.1^{b}	$-0.4 \pm 0.0^{a,b}$	

In each group of experiments, the effect of norepinephrine ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side) was first tested alone, and then, in the presence of tetrodotoxin (10^{-6} mol l^{-1} at the serosal side) or indomethacin (10^{-6} mol l^{-1} at the serosal side). Values are means ± S.E.M. The numbers in parenthesis give the number of tissues, in which a (1) phase of the norepinephrine response, i.e. an increase of Isc, was observed in relation to the total number of tissues investigated.

[a] $P < 0.05$ vs. baseline prior administration of norepinephrine.

[b] $P < 0.05$ vs. response to norepinephrine in the absence of any inhibitors.

h^{-1} cm^{-2} in the absence and 0.4 ± 0.1 μEq h^{-1} cm^{-2} in the presence of tetrodotoxin, $P < 0.05$, $n = 10$; Table 2). In the distal colon, the inhibition by the neurotoxin even amounted to 90% (Table 2), where norepinephrine induced a decrease in Isc of 1.0 ± 0.1 μEq h^{-1} cm^{-2} in the absence and only of 0.1 ± 0.1 μEq h^{-1} cm^{-2} in the presence of tetrodotoxin ($P < 0.05$, $n = 10$, Table 2).

A similar inhibition of the (2) phase of the catecholamine-induced current was observed with indomethacin, a blocker of the cyclooxygenase. Like tetrodotoxin, indomethacin (10^{-6} mol l^{-1} at the mucosal and the serosal side) induced a decrease in Isc (Table 1) and inhibited the (2) phase of the norepinephrine-effect (Table 2). In contrast to tetrodotoxin, indomethacin strongly inhibited the (1) phase of the response evoked by the catecholamine (Table 2).

3.2. Involvement of α-adrenoceptors

In order to characterize the adrenoceptors mediating the norepinephrine effect on Isc, antagonists of α-adrenoceptors were administered. The effects on the baseline Isc of all adrenergic antagonists are summarized in Table 1. Phentolamine (10^{-4} mol l^{-1} at the serosal side), which blocks both α_1- and α_2-adrenoceptors, did not inhibit the (1) phase of the norepinephrine response in either colonic segment, but rather, enhanced the increase in Isc evoked by the catecholamine, especially in the distal colon (Table 3). Furthermore, in the presence of this α-adrenoceptor

Table 3
Effect of adrenoceptor antagonists on norepinephrine-induced changes in Isc

	Proximal colon		Distal colon		*n*
	ΔIsc (μEq h^{-1} cm^{-2})				
	(1) Phase	(2) Phase	(1) Phase	(2) Phase	
Norepinephrine	0.3 ± 0.1^{a} (8/10)	-0.8 ± 0.1^{a}	0.2 ± 0.1^{a} (5/11)	-1.1 ± 0.2^{a}	10
+ phentolamine	0.4 ± 0.1^{a}	0.1 ± 0.1^{b}	$1.0 \pm 0.2^{a,b}$	-0.1 ± 0.1^{b}	11
Norepinephrine	0.1 ± 0.0^{a} (4/9)	-0.7 ± 0.1^{a}	0.1 ± 0.0^{a} (6/9)	-1.4 ± 0.3^{a}	9
+ prazosin	-0.1 ± 0.1	-0.3 ± 0.1	0.0 ± 0.1	-0.3 ± 0.1^{b}	
Norepinephrine	0.2 ± 0.1^{a} (5/6)	-0.2 ± 0.0^{a}	0.1 ± 0.0^{a} (5/8)	-0.7 ± 0.1^{a}	6–8
+ yohimbine	0.2 ± 0.1^{a}	0.0 ± 0.1^{b}	0.0 ± 0.0^{b}	$-0.1 \pm 0.0^{a,b}$	
Norepinephrine	0.1 ± 0.0^{a} (4/7)	-0.5 ± 0.1^{a}	0.1 ± 0.0^{a} (4/8)	-0.8 ± 0.1^{a}	7–8
+ propranolol	0.2 ± 0.0^{a}	-0.3 ± 0.1^{a}	0.1 ± 0.0^{a}	$-0.1 \pm 0.0^{a,b}$	
Norepinephrine	0.2 ± 0.0^{a} (8/10)	-0.7 ± 0.2^{a}	0.1 ± 0.0 (3/9)	-1.1 ± 0.1^{a}	9–10
+ atenolol	0.2 ± 0.1	-0.5 ± 0.1^{a}	0.1 ± 0.0^{a}	-0.2 ± 0.1^{b}	
Norepinephrine	0.2 ± 0.1^{a} (4/9)	-0.7 ± 0.1^{a}	0.1 (1/10)	-1.0 ± 0.2^{a}	9–10
+ ICI-118551	0.3 ± 0.0^{a}	-0.5 ± 0.1^{a}	0.0	$-0.1 \pm 0.0^{a,b}$	

In each group of experiments, the effect of norepinephrine ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side) was first tested alone, and then, in the presence of phentolamine (10^{-4} mol l^{-1} at the serosal side), prazosin (10^{-6} mol l^{-1} at the serosal side), yohimbine (10^{-5} mol l^{-1} at the serosal side), propranolol ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side), atenolol (10^{-4} mol l^{-1} at the serosal side), or ICI-118551 (10^{-5} mol l^{-1} at the serosal side). Values are means ± S.E.M. The numbers in parenthesis give the number of tissues, in which a (1) phase of the norepinephrine response, i.e. an increase of Isc, was observed in relation to the total number of tissues investigated.

[a] $P < 0.05$ vs. baseline prior administration of norepinephrine.

[b] $P < 0.05$ vs. response to norepinephrine in the absence of any inhibitors.

antagonist, an increase in Isc was observed in nearly all tissues. In contrast to the first phase, the (2) phase of the norepinephrine response was suppressed by this α-adrenoceptor antagonist (Table 3).

Prazosin (10^{-6} mol l^{-1} at the serosal side), an α_1-selective adrenoceptor antagonist, mimicked the inhibition of the (2) phase of the norepinephrine-induced current in the distal, but not in the proximal colon (Table 3). In contrast, the selective α_2-adrenoceptor antagonist, yohimbine (10^{-5} mol l^{-1} at the serosal side), nearly suppressed the (2) phase in both colonic segments (Table 3, Fig. 2). None of these antagonists mimicked the paradox action of phentolamine on the (1) phase of the norepinephrine response, i.e. the stimulation of the catecholamine-induced increase in Isc (Table 3).

3.3. β-Adrenergic effects of norepinephrine

Similar experiments were conducted with antagonists at β-adrenoceptors. Propranolol ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side), an antagonist which does not discriminate between the β_1- and β_2-subtype, had no effect on the (1) phase of the norepinephrine response in neither the proximal nor the distal colon (Table 3). The (2) phase of catecholamine-induced Isc was nearly suppressed in the distal colon. Norepinephrine evoked a decrease in Isc of 0.8 ± 0.1 μEq h^{-1} cm^{-2} in the absence and of only 0.1 ± 0.0 μEq h^{-1} cm^{-2} in the presence of propranolol ($P < 0.05$ vs. response in the absence of propranolol, $n = 8$; Table 3). In contrast to the distal compartment, propranolol was ineffective in the proximal colon (Table 3).

Surprisingly, both an antagonist at β_1-adrenoceptors, atenolol (10^{-4} mol l^{-1} at the serosal side), as well as an antagonist at β_2-adrenoceptors, ICI-118551 (10^{-5} mol l^{-1} at the serosal side), mimicked the inhibition of the (2) phase of the norepinephrine-response by propranolol in the distal colon (Table 3, Fig. 2). As had to be expected, neither drug had any effect on the (1) phase of the catecholamine-induced current in the two colonic segments nor on the (2) phase in the proximal colon (Table 3, Fig. 2).

3.4. Involvement of β_3-adrenoceptors

None of the antagonists at α- or β-adrenoceptors used in the preceding experiments did inhibit the (1) phase of the catecholamine response. Therefore, in the next set of experiments, drugs acting at β_3-adrenoceptors were applied. Indeed, BRL 37344 (10^{-5} mol l^{-1} at the serosal side), a selective agonist at β_3-adrenoceptors, mimicked the early action of norepinephrine. BRL 37344 induced an

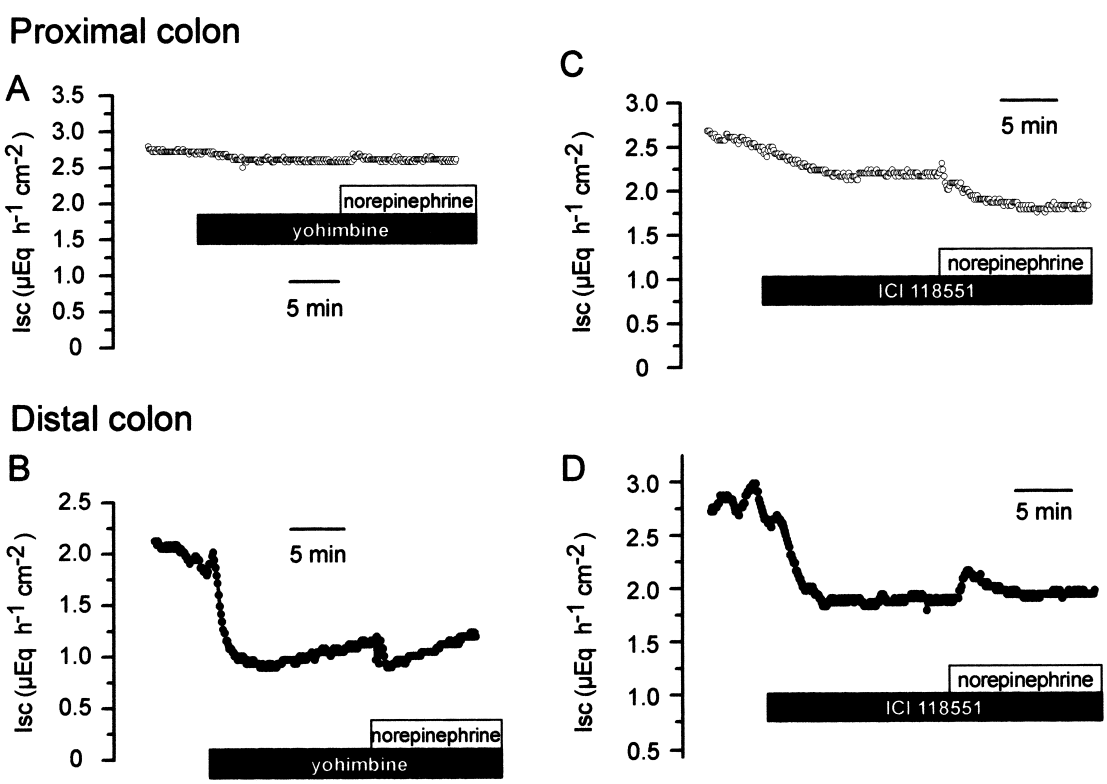

Fig. 2. Effect of two adrenoceptor antagonists on the Isc response evoked by norepinephrine ($5 \cdot 10^{-6}$ mol $\cdot l^{-1}$ at the serosal side), **A, B**: Effect of the α_2-adrenoceptor antagonist yohimbine (10^{-5} mol $\cdot l^{-1}$ at the serosal side) in the proximal (A) and distal colon (B). **C, D**: Effect of the β_2-adrenoceptor antagonist ICI-118551 (10^{-5} mol $\cdot l^{-1}$ at the serosal side) in the proximal (C) and the distal colon (D). The tracings are typical for 6–10 experiments with each colonic segment; for statistics, see Table 3.

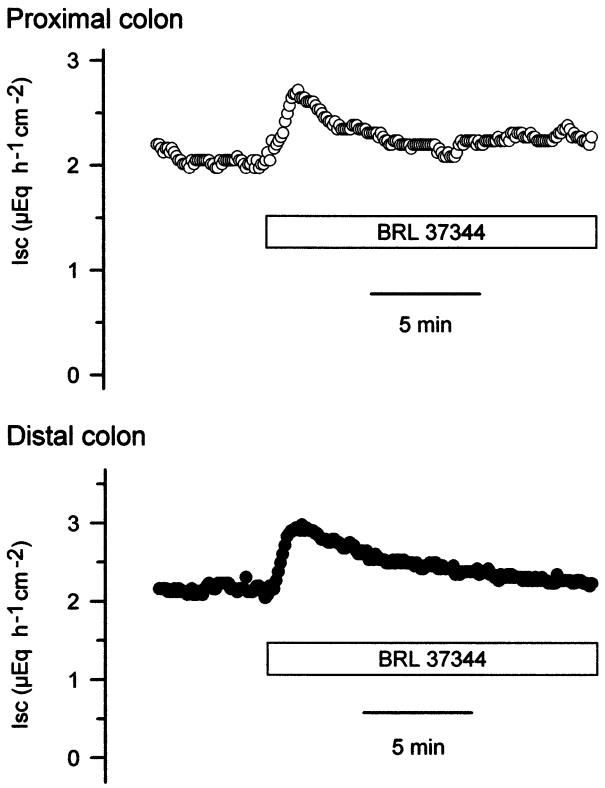

Fig. 3. Effect of the β_3-adrenoceptor agonist BRL 37344 (10^{-5} mol·l^{-1} at the serosal side) in the proximal (open circles) and distal (filled circles) colon. The tracings are representative for 10–12 experiments with each colonic tissue. In both colonic segments, BRL 37344 evoked an increase in Isc of 0.2 ± 0.1 μEq·h^{-1}·cm^{-2} ($P < 0.05$; $n = 10$–12).

increase in Isc of 0.2 ± 0.1 μEq h^{-1} cm^{-2} ($P < 0.05$, observed in 10 of 10 tissues tested; Fig. 3) in the proximal and of 0.2 ± 0.1 μEq h^{-1} cm^{-2} ($P < 0.05$, observed in nine of 12 tissues tested; Fig. 3) in the distal colon. In contrast to the native catecholamine, BRL 37344 evoked only an increase in Isc, which was never followed by a decrease; instead, the current decayed slowly within 10–15 min to the former baseline. When Cl^- was substituted by the impermeable anion, gluconate, the increase in Isc induced by BRL 37344 was reduced by 50% in the proximal colon, i.e. the agonist induced only an increase in Isc by 0.1 ± 0.0 μEq h^{-1} cm^{-2} observed in seven out of eight tissues. In the distal colon, the agonist had no more significant effect on Isc, when Cl^- ions were replaced ($n = 8$).

The non-conventional partial β_3-agonist CGP 12177 (10^{-6} mol l^{-1} at the serosal side), an adrenoceptor blocking agent that exhibits agonist effects at concentrations considerably greater than those causing blockade of the β_1- or β_2-adrenoceptors (Kaumann and Molenaar, 1996; Strosberg, 1997), mimicked the effect of norepinephrine. The drug induced a significant transient increase in Isc of 0.2 ± 0.1 μEq h^{-1} cm^{-2} in seven out of 11 tissues in the proximal and 0.2 ± 0.0 μEq h^{-1} cm^{-2} in five out of 16 tissues in the distal colon ($P < 0.05$, $n = 11$–16), followed by a long-lasting decrease in both colonic segments (data not shown).

Finally, it was tested, whether antagonists of β_3-adrenoceptor might be able to prevent the stimulation of Isc during the first phase of the epinephrine response. Preincubation of the tissue with bupranolol (10^{-5} mol l^{-1} at the serosal side), a non-selective β-adrenoceptor antagonist with a high affinity for β_3-adrenoceptor (Alexander and Peters, 1999), completely prevented the (1) phase of the norepinephrine-induced current (Table 4). A similar inhibition was observed with SR 59230A (10^{-5} mol l^{-1} at the serosal side), a selective β_3-adrenoceptor antagonist (Alexander and Peters, 1999), at least in the proximal colon. In the absence of SR 59230A, the norepinephrine-induced Isc amounted to 0.2 ± 0.0 μEq h^{-1} cm^{-2} in seven out of 11 tissues, whereas in the presence of the blocker, norepinephrine did not evoke an increase in Isc at all ($P < 0.05$ vs. absence of SR 59230A, $n = 11$; Table 4). In the distal colon, SR 59230A was ineffective (Table 4). A similar pattern of inhibition was observed, when the ability of phentolamine to enhance the initial phase of the catecholamine-stimulated current was used in order to increase the frequency, at which norepinephrine induced the (1) phase. If the tissues were pretreated with phentolamine, norepinephrine induced constantly an increase in Isc in all tissues investigated. Again, in the presence of

Table 4
Effect of β_3-adrenoceptor antagonists on the norepinephrine-induced Isc

	Proximal colon	Distal colon	*n*
	(1) Phase (ΔIsc in μEq h^{-1} cm^{-2})		
Norepinephrine	0.2 ± 0.0^a (7/9)	0.3 ± 0.1^a (5/10)	9–10
+ bupranolol	-0.1 ± 0.0^b	-0.0 ± 0.0^b	
Norepinephrine	0.2 ± 0.0^a (7/11)	0.1 ± 0.0^a (6/10)	10–11
+ SR 59230A	-0.2 ± 0.1^b	0.1 ± 0.1	
In the presence of phentolamine			
Norepinephrine	0.3 ± 0.1^a (9/9)	0.2 ± 0.2^a (14/14)	9–14
+ bupranolol	$0.1 \pm 0.0^{a,b}$	$0.1 \pm 0.0^{a,b}$	
Norepinephrine	0.3 ± 0.1^a (8/8)	0.5 ± 0.1^a (9/9)	8–9
+ SR 59230A	$0.1 \pm 0.0^{a,b}$	0.3 ± 0.1^a	

In the two upper groups of experiments, the effect of norepinephrine ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side) was first tested alone, and then, in the presence of bupranolol (10^{-5} mol l^{-1} at the serosal side), or SR 59230A (10^{-5} mol l^{-1} at the serosal side). In the two lower groups of experiments, the effect of norepinephrine ($5 \cdot 10^{-6}$ mol l^{-1} at the serosal side) was first tested in the presence of phentolamine (10^{-4} mol l^{-1} at the serosal side) to increase the occurrence of the (1) phase of the catecholamine response, and then, in the combined presence of phentolamine, norepinephrine and bupranolol or SR 59230A, respectively. Values are means ± S.E.M. The numbers in parenthesis give the number of tissues, in which a (1) phase of the norepinephrine response, i.e. an increase of Isc, was observed in relation to the total number of tissues investigated.

[a] $P < 0.05$ vs. baseline prior administration of norepinephrine.

[b] $P < 0.05$ vs. effect of norepinephrine in the absence any inhibitor (two upper groups), or in the sole presence of phentolamine (two lower groups).

phentolamine, bupranolol inhibited the (1) phase of the Isc response in both colonic segments, whereas SR 59230A had only a significant inhibitory effect in the proximal colon (Table 4).

4. Discussion

In the present study, the effect of norepinephrine on Isc in the rat colon was investigated. The catecholamine induced a biphasic change in Isc, i.e. a first transient increase, which reversed to a long-lasting decrease in both colonic segments. These effects resemble those of epinephrine with regard to the time course and the amplitude of the current changes (Hörger et al., 1998). A further common property is the high variability, at which both catecholamines evoke an initial increase in Isc, which made statistical examinations of the effect of putative inhibitors difficult. However, when norepinephrine was administered three times to the same tissue in the absence of any inhibitors, nearly 90% of the tissues, which exhibited a transient increase in Isc during the first administration, also exhibited such an increase during the second and third administration. Therefore, it is quite unlikely that profound effects, such as the observation that e.g. bupranolol completely prevented the (1) phase of the norepinephrine-induced current (Table 4), can be explained simply by the biological variability of the catecholamine response. In contrast to the highly variable transient increase in Isc, the delayed, secondary decrease is nearly always observed.

A transient increase in Isc has up to now been observed in the rat colon (Racusen and Binder, 1979; Hörger et al., 1998) and the guinea-pig distal colon (Rechkemmer et al., 1996), but not, e.g. in the rabbit ileum (Field and McColl, 1973) or rabbit colon (Smith and McCabe, 1986). Anion substitution experiments and the sensitivity against bumetanide indicated that in the rat colon, the increase in Isc, evoked by epinephrine, is caused by a Cl^- secretion (Hörger et al., 1998). As the current responses induced by norepinephrine and epinephrine are nearly identical, it seems reasonable to assume that also the (1) phase of the electrogenic effect of norepinephrine is induced by a Cl^- secretion.

One aim of the present study was to characterize the adrenoceptor mediating this anion secretion during the initial phase of the responses induced by catecholamines. As previously demonstrated for epinephrine (Hörger et al., 1998), also the norepinephrine-induced increase in Isc was resistant against classical α- and β-adrenoceptor antagonists, e.g. phentolamine or propranolol (Table 3). A similar resistance in other tissues, e.g. fat tissue, intestinal smooth muscle or the heart (for references see Strosberg, 1997), led to the idea that a β_3-adrenoceptor might be involved. Indeed, BRL 37344, a selective β_3-adrenoceptor agonist, mimicked the (1) phase of the catecholamine response (Fig. 3). Vice versa, the specific β_3-adrenoceptor antagonist SR 59230A inhibited the norepinephrine-induced increase in Isc at least in the proximal colon, whereas the non-selective β-adrenoceptor antagonist bupranolol was effective in both colonic segments (Table 4). Taken together, these data indicate the presence of a β_3-adrenoceptor in the rat colon.

The putative β_3-adrenoceptor is probably not located directly at the epithelial cells responsible for anion secretion since the cyclooxygenase inhibitor, indomethacin, suppressed the (1) phase of the Isc evoked by both epinephrine (Hörger et al., 1998), as well as norepinephrine (Table 2). Consequently, subepithelial cells, which are responsible for the endogenous production of prostaglandins (Craven and DeRubertis, 1986), seem to be the primary target for this action of the catecholamines. These cells seem to be under the inhibitory control of enteric neurons, which was abolished by the neurotoxin, tetrodotoxin, as well as by the α-adrenoceptor antagonist, phentolamine (see Hörger et al., 1998 for epinephrine and Table 3 for norepinephrine).

The increase in Isc was always followed by a (2) phase, in which the Isc decreased below the former baseline. In rat colon (Hörger et al., 1998), rabbit colon (Smith and McCabe, 1986) and guinea-pig colon (Rechkemmer et al., 1996), unidirectional flux experiments have revealed that in the case of epinephrine, the change in Isc is caused by K^+ secretion. Due to the strong similarities between the Isc induced by epinephrine and that induced by norepinephrine (see above), most probably, the (2) phase of the electrical response evoked by norepinephrine represents a K^+ secretion.

In the proximal colon, norepinephrine (Table 3) and epinephrine (Hörger et al., 1998) activate K^+ secretion via stimulation of an α_2-adrenoceptor, whereas some differences between the action of both catecholamines emerged in the distal colon. In the latter colonic segment, epinephrine induces K^+ secretion via a β_2-adrenoceptor (Hörger et al., 1998), whereas the (2) phase of the Isc induced by norepinephrine is mediated both by α- and β-subtypes (Table 3). Surprisingly, both α-, as well as β-adrenoceptor antagonists alone, inhibited already 80–90% of the current during the second phase. A reasonable explanation for this finding may be the known potentiation of different intracellular signaling pathways during the induction of secretion. For example, to stimulate a maximal Cl^- secretion, it is necessary to open apical Cl^- channels via cAMP-dependent phosphorylation, as well as to open basolateral Ca^{2+}-dependent K^+ channels to maintain the driving force for anion exit out of the cell (Strabel and Diener, 1995). Both intracellular pathways are also involved in the regulation of K^+ transport (Diener et al., 1996; Schultheiss and Diener, 1997; Heinke et al., 1998). As β-adrenoceptors are generally positively coupled to the adenylate cyclase and stimulation of α-adrenoceptors often leads to an increase in the intracellular Ca^{2+} concentration

(for review see e.g. Lefkowitz et al., 1990), a similar synergistic interaction between both pathways may be well thought of to explain the sensitivity of the norepinephrine to both α- and β-adrenoceptor blocking drugs.

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