*Journal of Physiology* (1991), **437**, *pp.* 341–350
*With 6 figures*
*Printed in Great Britain*



# NEUROKININ A INCREASES SHORT-CIRCUIT CURRENT ACROSS RAT COLONIC MUCOSA: A ROLE FOR VASOACTIVE INTESTINAL POLYPEPTIDE

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(*Received 4 October 1990*)

## SUMMARY

1. Neurokinin A (NKA) is a mammalian tachykinin distributed principally in the nervous system, including the myenteric innervation of the gut.

2. NKA may be involved in neurogenic inflammation and as a modulatory factor in the diarrhoea associated with mucosal inflammation of inflammatory bowel disease (ulcerative colitis).

3. We evaluated the effect of NKA on the short-circuit current $I_{sc}$, assumed to reflect electrogenic chloride secretion, across muscle-stripped rat colonic mucosa mounted in Ussing chambers.

4. Serosal addition of NKA produced a concentration-dependent (0·1–100 nM) increase in $I_{sc}$ with an $EC_{50}$ (half-maximal effective concentration) value of 7·5 nM. The maximum (mean ± S.E.M.) increase in $I_{sc}$ ($\mu A/cm^2$) for NKA was 111 ± 10.

5. Tetrodotoxin (0·5 $\mu$M) and bumetanide (10 $\mu$M), but not atropine (1·0 $\mu$M), hexamethonium (100 $\mu$M) or pyrilamine (10 $\mu$M), significantly inhibited NKA-induced increases in $I_{sc}$.

6. The response to NKA was attenuated by 45 min pre-treatment with antisera raised against vasoactive intestinal polypeptide (VIP). Moreover, prior desensitization to VIP attenuated the effect of NKA.

7. These studies suggest that NKA increases $I_{sc}$ in rat colon, in part, through a non-cholinergic neural mechanism involving VIP.

## INTRODUCTION

Neurokinin A (NKA, substance k, neuromedin L) is a mammalian tachykinin (Kangawa, Minamino, Fukuda & Matsuo, 1983; Kimura, Okada, Sugita, Kanagawa & Manekata, 1983), derived from $\beta$-preprotachykinin A and $\gamma$-preprotachykinin A (Maggio, 1988). It shares a common C-terminus and co-exists with other tachykinins

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in the same subpopulation of neurons, and therefore may also act as a neurotransmitter (Minamino, Kangawa, Fukuda & Matsuo, 1984; Dalsgaard, Haegerstrand, Theodorsson-Norheim, Brodin & Hokfelt, 1985; Dockray, 1987).

Neurogenic inflammation, characterized by vasodilatation, plasma extravasation and smooth muscle contraction initiated by release of proinflammatory mediators from sensory unmyelinated afferent nerve endings, may be a contributing factor in the pathophysiology of inflammatory bowel disease (Mayer, Raybould & Koelbel, 1988). Neurokinin A is a candidate mediator in neurogenic inflammation. It is localized in sensory neurons (Hua, Theodorsson-Norheim, Brodin, Lundberg & Hokfelt, 1985), increases vascular permeability and is a vasodilator (Foreman, 1987). Receptor binding sites for neurokinin A are expressed by cells mediating inflammatory and immune responses (Mantyh, Mantyh, Gates, Virna & Maggio, 1988). Tachykinins may affect inflammation by increasing mast cell secretion, promoting monocyte chemotaxis and stimulating phagocytosis and lysosomal enzyme release by neutrophils (Shanahan & Anton, 1988).

Recently, NKA was shown under short-circuit conditions to cause net electrolyte secretion in the canine tracheal epithelium (Rangachari, McWade & Donoff, 1987; Tamaoki, Ueki, Widdicombe & Nadel, 1988) and the guinea-pig jejunum (Mathison & Davidson, 1989). Since the colonic mucosal secretory response to NKA has not been reported, and secretory diarrhoea is associated with ulcerative colitis, we investigated the effects and mechanism of action of NKA on rat colonic mucosa *in vitro*.

Part of this work has been presented in abstract form (Tien, Wallace & Gaginella, 1988).

## METHODS

### *Tissue preparation*

Male Sprague-Dawley rats (250–400 g) were killed by cervical dislocation, and the distal colon was excised. The colon was opened and rinsed with ice-cold saline solution (0·9% w/v NaCl). The circular and longitudinal muscle layers were stripped off with a pair of fine forceps. Pieces of muscle-stripped mucosa were mounted as flat sheets in Ussing chambers (0·64 $cm^2$) and bathed with buffered physiological saline solution composed of (mM): NaCl, 120·2; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaH_2PO_4$, 1·2; $NaHCO_3$, 25; glucose, 11·1 (pH 7·4, 37 °C), gassed with 95% $O_2$ and 5% $CO_2$.

### *Electrical measurements*

Electrical potential difference (PD) was monitored at the beginning of each experiment with a pair of saturated KCl–agar (3%) bridges placed on each side of the tissue. The circuit was completed with calomel half-cell electrodes connected to a DVC 1000 voltage clamp (WPI Instruments, New Haven, CT, USA). Short-circuit current ($I_{sc}$) was applied through silver–silver chloride electrodes to the tissue to clamp open-circuit PD (potential difference) at zero, and compensation was made for fluid resistance. Initial tissue conductance ($G_t$) was calculated from the values of $I_{sc}$ and PD according to Ohm's law. The reciprocal of $G_t$ (mS/$cm^2$) times 1000 equals the tissue resistance ($R$, Ω $cm^2$).

### *Concentration–response curves*

After mounting, the tissues were allowed to equilibrate for at least 30–60 min; effects on presumed electrogenic chloride secretion were monitored as changes in $I_{sc}$. The concentration required to increase $I_{sc}$ by 50% ($EC_{50}$) was calculated from the curves by non-linear least-squares regression.

*Effects of antagonists*

Antagonists were added to the serosal side of the tissue for various times (see individual drugs below) prior to agonist addition. If blockade was observed, bethanechol (0·4 mM) or aminophylline (20 mM) was added after the agonist response to ensure that antagonism, rather than poor tissue viability, was responsible for the lack of response. Antagonist controls were conducted on a separate tissue from the same animals.

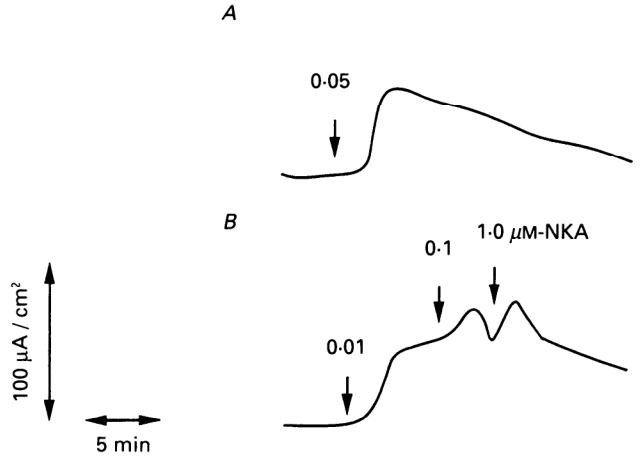


Fig. 1. Representative effect of NKA (μM) on $I_{sc}$ after a single concentration (*A*) and following cumulative addition (*B*).

*VIP antisera studies*

VIP antisera was used to assess a role for VIP in the response to NKA. The VIP antiserum was used at a final dilution of 1 : 200. Each tissue preparation treated with VIP antiserum was compared with a control tissue from the same animal. Basal $I_{sc}$ values and conductance were comparable in VIP-antisera treated and control tissues.

*Materials*

NKA, VIP, TTX, hexamethonium and pyrilamine were obtained from Sigma (St Louis, MO, USA), and bumetanide was obtained from Hoffmann-La Roche Inc., Nutley, NJ, USA. Agents not soluble in distilled water or saline were dissolved in dimethyl sulphoxide (DMSO). Equivalent amounts of DMSO (without drug) added to the serosal side did not affect $I_{sc}$. VIP antisera (Ras 71651-N, rabbit) was purchased from Penninsula Laboratories, San Carlos, CA, USA.

*Statistical analysis*

All values are expressed as the means ± S.E.M. The Student's paired or unpaired *t* test was used for testing statistical significance.

## RESULTS

### *Effect of NKA on $I_{sc}$*

NKA evoked a rapid but transient increase in $I_{sc}$ upon serosal addition (Fig. 1). The responses were concentration dependent over a range of 0·5–100 nM (Fig. 2); the $EC_{50}$ value was determined to be 7·5 nM. The maximum response to NKA was $111 \pm 10$ μA/cm² ($n = 26$ tissues from thirteen animals).

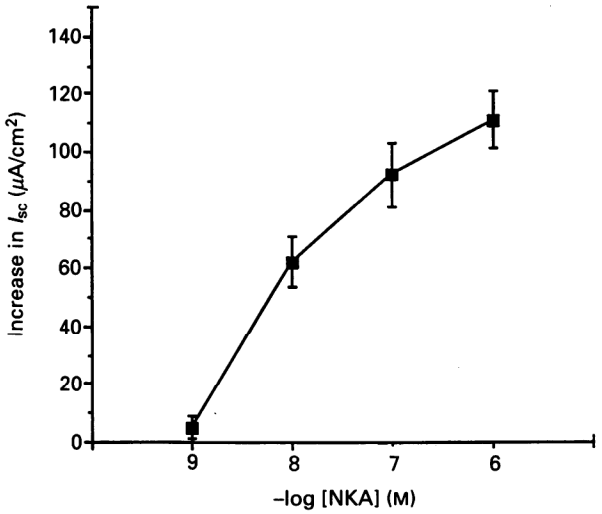


Fig. 2. Concentration–response curve (serosal addition) for NKA-induced increases $I_{sc}$ across rat colonic mucosa. Results are expressed as the change in $I_{sc}$ ($\mu$A/cm$^2$) above baseline *vs.* log concentration of NKA. Symbols and bars represent the means ± S.E.M. of determinations for at least twelve animals. The maximal increase in $I_{sc}$ was achieved at $10^{-7}$–$10^{-6}$ M.

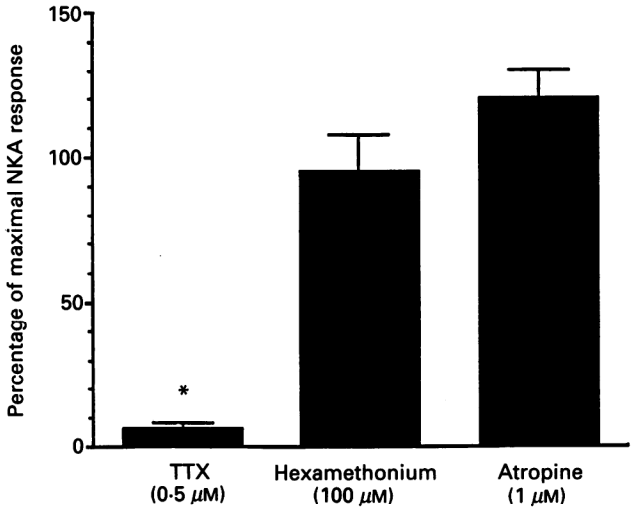


Fig. 3. Effects of tetrodotoxin (TTX), atropine and hexamethonium against NKA (50 nM). Inhibitors were added 10 min before NKA. Results are expressed as means ± S.E.M. of three to eight animals. *$P < 0{\cdot}05$.

*Effects of inhibitors*

To substantiate that the NKA-evoked increase in $I_{sc}$ was due to active chloride ion secretion, tissues were incubated with bumetanide (10 $\mu$M, 30 min) before adding NKA to the tissue bath. Responses to a maximally effective concentration (50 nM)

of NKA were significantly ($P < 0{\cdot}001$) reduced by serosal addition of bumetanide to $14 \pm 4\%$ of control. Unidirectional flux measurements with $^{36}Cl^-$ could not be conducted due to the transient nature of the response.

TTX (0·5 $\mu$M) inhibited by 93% the $I_{sc}$ response to 50 nM-NKA (Fig. 3). Atropine (1 $\mu$M, 10 min), which completely inhibited the response to 0·4 mM-bethanechol, had

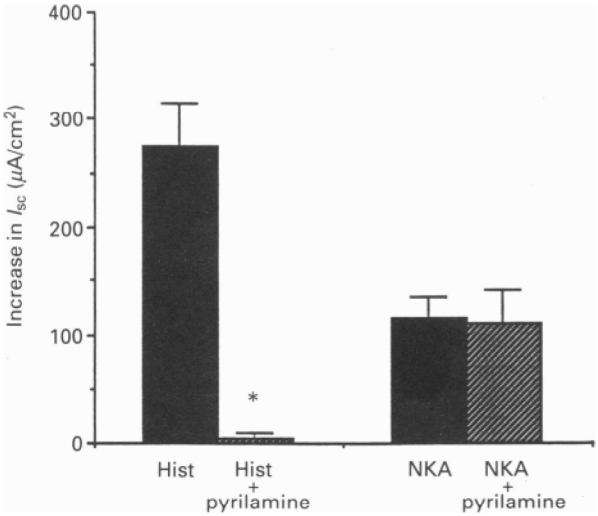


Fig. 4. Effects of the $H_1$ receptor antagonist pyrilamine (10 $\mu$M) against 1 mM-histamine and NKA (50 nM). The antagonist was added 10 min prior to agonists. All drugs were added to the serosal side. Data are expressed as means ± S.E.M. for determinations from at least six animals. *$P < 0{\cdot}001$.

no significant effect on responses elicited by NKA; the ganglionic blocker, hexamethonium, also failed to inhibit the response to NKA (Fig. 1).

Because other neurokinins are reported to release histamine from mast cells (Erjavec, Lembeck, Florjanc-Irman, Skofttsch, Donnerer, Saria & Holzer, 1981; Foreman, 1987), the histamine receptor $H_1$ antagonist, pyrilamine, was used to determine if it would block the NKA-induced response. Pyrilamine (10 $\mu$M, 15 min) significantly blocked the histamine (1 mM) but not the NKA (50 nM) response (Fig. 4).

The effect of VIP antisera on the $I_{sc}$ response to NKA was examined. A dilution of 1:200 attenuated both the NKA response (Fig. 5*A*) and the response to VIP (Fig. 5*B*). However, pre-treatment with non-specific rabbit serum (1:200) or pre-neutralized VIP antiserum did not affect the response to NKA.

*Desensitization studies*

After stimulation of the tissues with NKA, subsequent additions of the same concentration of the peptide failed to produce a response, indicating homologous

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