Effect of $Na^+,K^+,2Cl^-$ Cotransport Inhibitor Bumetanide on Electrical and Contractile Activity of Smooth Muscle Cells in Guinea Pig Ureter

I. V. Kovalev, M. B. Baskakov, Ya. D. Anfinogenova, Yu. L. Borodin, A. A. Kilin, I. L. Minochenko, A. G. Popov, L. V. Kapilevich, M. A. Medvedev*, and S. N. Orlov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 8, pp. 167-172, August, 2003
Original article submitted September 17, 2002

The effect of $Na^+,K^+,2Cl^-$ cotransport inhibitor bumetanide on action potentials and contractions of smooth muscle cells in the ureter of guinea pigs evoked by electrical stimulation was studied by the method of double sucrose bridge. Bumetanide (10-100 μM) dose-dependently suppressed action potential and contractions of smooth muscle cells induced by 1-10 μM histamine, 10 μM mesatone, 5 mM tetraethylammonium, and 100 μM sodium nitroprusside. Our findings suggest that test substances modulate $Na^+,K^+,2Cl^-$ cotransport in smooth muscle cells.

Key Words: *bumetanide; $Na^+,K^+,2Cl^-$ cotransport; biologically active substances; smooth muscle cells*

The presence of $Na^+,K^+,2Cl^-$ cotransport in smooth muscle (SMC) cells is well established [11-13]. This mechanism attracts much recent attention, since it plays an important role in volume-dependent processes [7,8,10-12]. Moreover, $Na^+,K^+,2Cl^-$ cotransport contributes to the contractile effects of biologically active substances (BAS), relaxants, and nitrocompounds in SMC of vessels sensitive to hyperosmotic factors [6-8]. However, the role of $Na^+,K^+,2Cl^-$ cotransport in electrogenesis of SMC remains unclear.

MATERIALS AND METHODS

We examined smooth muscle segments of the ureter (10-12 mm) isolated from guinea pigs.

Action potentials (AP) and SMC contractions were evoked by electrical stimulation and studied by the method of double sucrose bridge [1,2]. AP were recorded with nonpolarized electrodes. Contractile activity was determined on a 6MKh2B mechanotron under near-isometric conditions. Parameters of electrical and contractile activity of SMC were amplified, transferred to an analog-digital transducer, and recorded on an IBM PC.

We used Krebs solution containing 133 mM NaCl, 5.0 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 15 mM tris(hydroxymethyl)aminomethane, and 11.5 mM glucose (pH 7.35) and benzene-sodium solution with equimolar substitution of NaCl for choline chloride. Histamine, mesatone (Reakhim), sodium nitroprusside (NP), bumetanide (Sigma), and tetraethylammonium chloride (Serva) were added to Krebs solution. The temperature was maintained at 36.8-37.0°C.

The results were analyzed by Student's *t* test. The amplitude of the unelectrotonic potential (UEP), parameters of AP (peak amplitude and duration of the plateau phase), and amplitude of contraction in Krebs solution or in the presence of test substances in response to electrical stimulation were taken as the control (100%).

RESULTS

For evaluation of the mechanisms of $Na^+,K^+,2Cl^-$ cotransport in SMC we used bumetanide acting as a

Department of Biophysics, *Department of Normal Physiology, Siberian Medical University, Tomsk; **Research Center, University of Montreal, Canada. ***Address for correspondence:*** Kovalev@mail.ru. Kovalev I. V.

0007-4888/03/1362-0145$25.00

selective inhibitor of this ion-transporting system [3,7, 8,11,12]. Bumetanide (10 μM) added to Krebs solution for 8-10 min reduced the strength of contractions during membrane hyperpolarization and slightly decreased AP in ureteral SMC (Fig. 1, *a*). Increasing the concentration of bumetanide to 50-100 μM abolished inhibition of contractions.

Bumetanide had no effect on AP of ureteral SMC. This substance probably inhibits electroneutral ion cotransport, whose basal activity is low [3,11,12]. The inhibition of $Na^+,K^+,2Cl^-$ cotransport can affect inward chlorine current [3,6,9]. Blockade of outward chlorine current during passive redistribution typical of smooth muscles [5] can contribute to membrane hyperpolarization sufficient for the decrease in contractile activity of ureteral SMC. Published data show that bumetanide in a concentration of 200 μM inhibits K^+,Cl^- cotransport in epitheliocytes [3,9]. In SMC this cotransport was also found [11,13], but it is insensitive to bumetanide [6,11,12].

Suppression of contractions during membrane hyperpolarization in SMC can be explained by increased K^+ conductance [2,4,5]. The role of this process in the effect of bumetanide was studied in experiments with the K^+ channel blocker tetraethylammonium (TEA) [2,4,5]. TEA in a concentration of 5 mM abolished the increase in AP and inhibition of K^+ conductance in SMC membranes produced by bumetanide (100%, Fig. 1, *b*, Table 1).

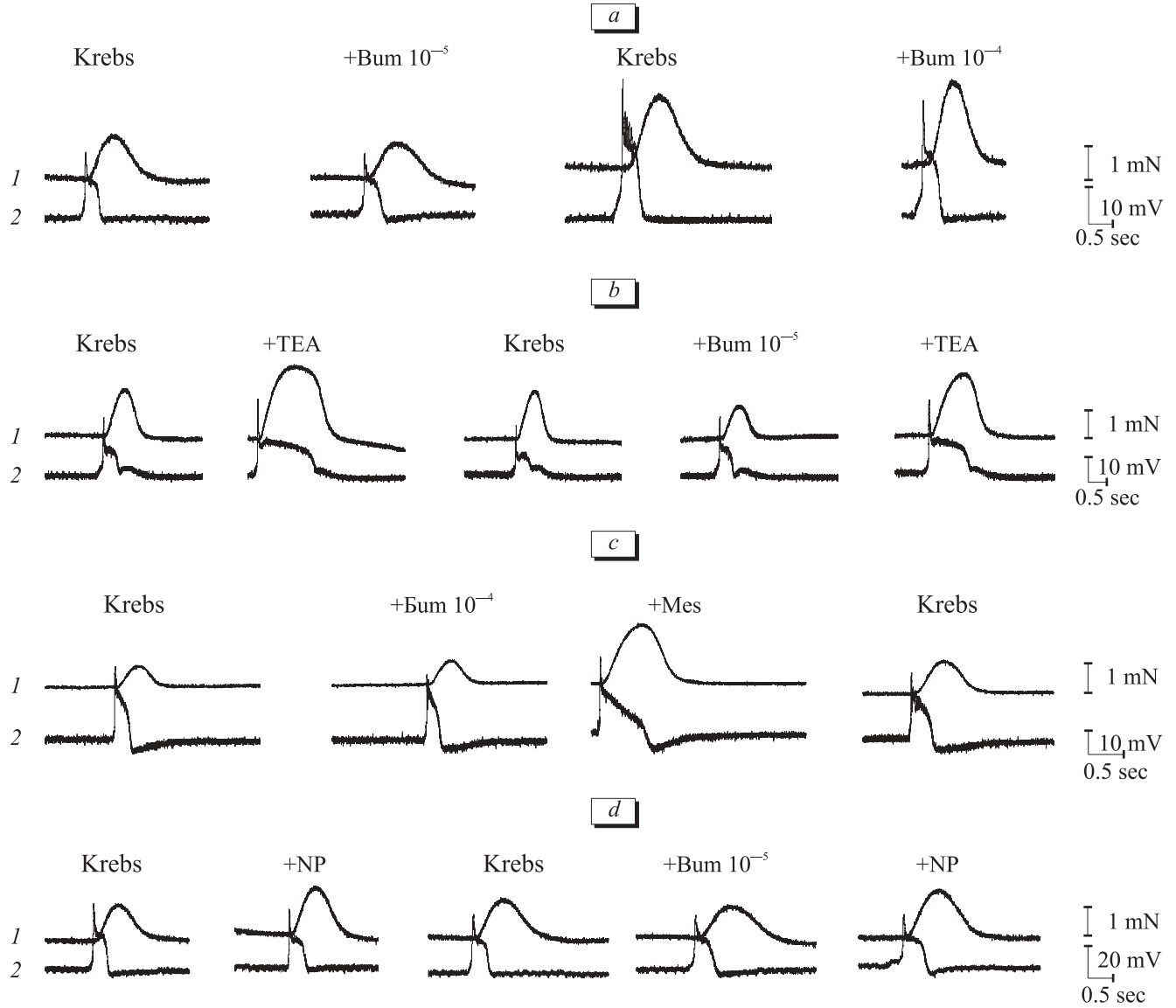

Fig. 1. Effect of bioactive substances (BAS) on the influence of bumetanide on contractile (*1*) and electrical activity (*2*) of ureteral SMC in guinea pigs. *a*) 12-15 min after addition of bumetanide in concentrations of 10 and 100 μM (+Bum); *b*) 5 mM tetraethylammonium (+TEA); *c*) addition of mesatone in a concentration of 10 μM (+Mes); *d*) 100 μM sodium nitroprusside (+NP). Here and in Fig. 2: calibration signal and reference time are shown on the right side.

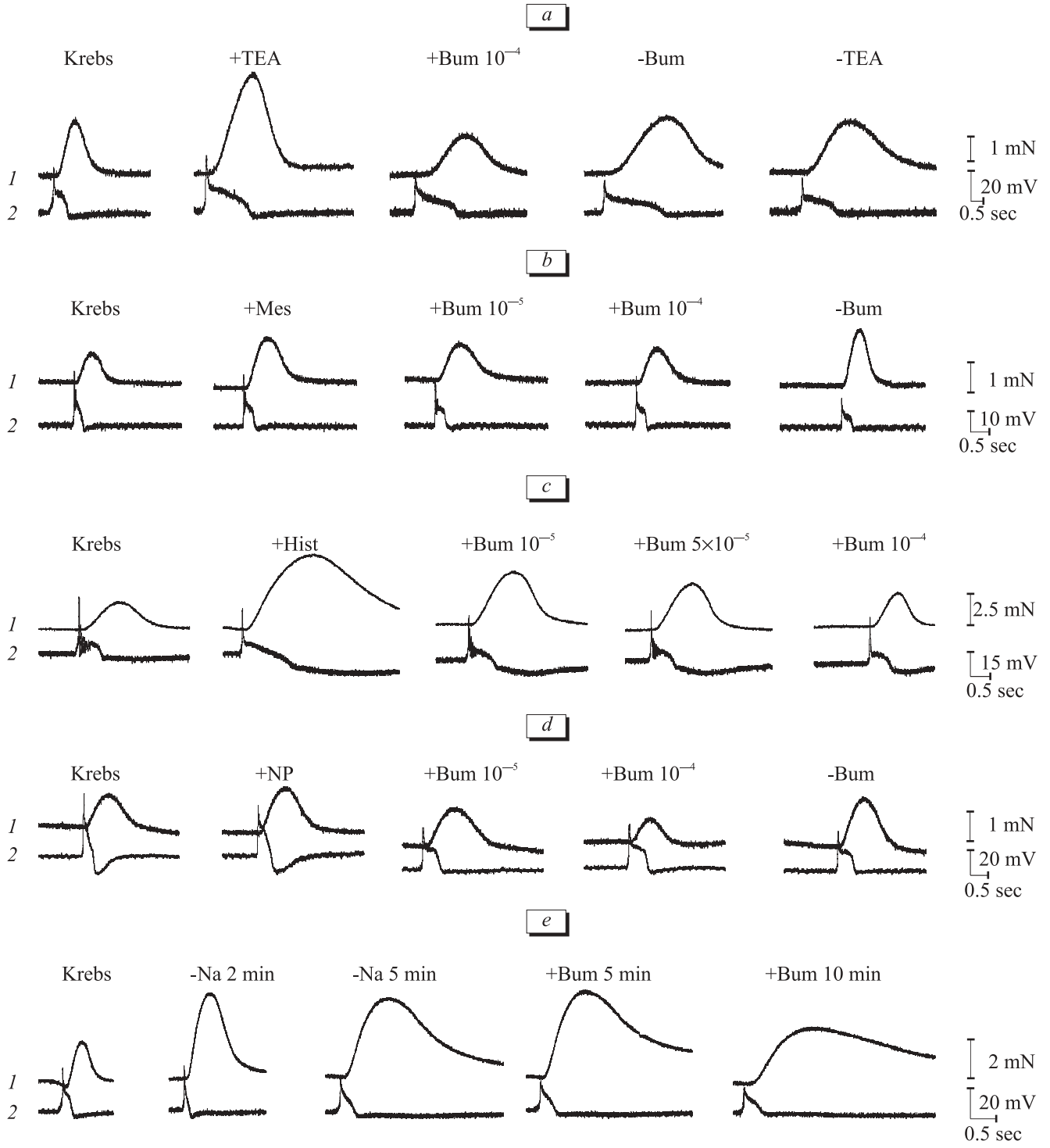

Fig. 2. Effect of bumetanide on the influence of BAS on contractile (*1*) and electrical activity (*2*) of ureteral SMC in guinea pigs. *a*) Effect of 100 μM bumetanide (+Bum) on 5-min exposure to 5 mM tetraethylammonium (+TEA); *b*) effect of bumetanide in concentrations of 10 and 100 μM (+Bum) on 5-min exposure to 10 μM mesatone (+Mes); *c*) after treatment with 10 μM histamine (+Hist); *d*) after treatment with 100 μM sodium nitroprusside (+NP); *e*) effect of 10 μM bumetanide (+Bum) in sodium-free Krebs solution (-Na) after 5- and 10-min exposure.

Bumetanide dose-dependently decreased the duration of AP and inhibited contractions of ureteral SMC during blockade of K^+ conductance (Fig. 2, *a*). It should be emphasized that electrical and contractile activities were below the control (Table 2). These data suggest that inhibition of $Na^+,K^+,2Cl^-$ cotransport by bumetanide did not potentiate, but reduced K^+ conductance of SMC membranes.

Previous studies showed that bumetanide can block not only outward, but also inward currents in SMC

TABLE 1. Effect of BAS on Amplitude of UEP, Duration (Plateau Phase), Amplitude of AP, and Amplitude of Contractions in Ureteral SMC of Guinea Pigs

Substance	UEP	AP amplitude	Duration (plateau phase)	Amplitude of contraction
TEA, 5 mM (*n*=17)	109.8±14.7	121.4±9.6*	216.0±33.3*	157.0±26.3*
Mesatone, 10 μM (*n*=16)	97.9±15.0	95.3±9.6	151.0±18.8*	188.0±28.1*
Histamine, 1 μM (100%, *n*=8)	100.24±14.70	93.5±4.8**	116.4±11.7**	132.8±16.2**
Histamine, 10 μM (*n*=11)	96.7±33.7	82.6±7.3*	161.3±25.1*	203.7±23.3*
NP, 100 μM (*n*=11)	88.1±10.2**	93.0±16.1	107.7±20.1	124.6±9.3*

Note. Control (100%): effect of Krebs solution. Here and in Fig. 2: *n*, number of experiments. *$p<0.01$ and **$p<0.05$ compared to the control.

(*e.g.*, Ca^{2+}) [7]. Moreover, TEA suppresses Ca^{2+}-dependent components of membrane K^+ conductance [2,4,5]. When such is the case, membranes of ureteral SMC can "escape" the effect of the K^+-channel blocker. This process coincides with blockade of Ca^{2+} fluxes with bumetanide.

Ca^{2+} entry is a possible target for the action of bumetanide [7]. It can be revealed after the addition of BAS activating Ca^{2+} channels. Pretreatment of ureteral SMC with bumetanide facilitated the activating effect of the α_1-sympathomimetic mesatone on SMC (Fig. 1, *c*). These changes were previously observed

TABLE 2. Effect of Bumetanide in Various Concentrations and Influence of BAS on the Amplitude of UEP, Duration (Plateau Phase), Amplitude of AP, and Amplitude of Contractions in Ureteral SMC of Guinea Pigs

Bumetanide concentration		Krebs solution	TEA, 5 mM	Mesatone, 10 μM	Histamine		NP, 100 μM
					1 μM	10 μM	
10 μM	UEP	101.5±23.1 (*n*=9)	92.5±38.4 (*n*=6)	100.1±13.1 (*n*=5)	99.8±11.0 (*n*=8)	111.7±11.0 (*n*=9)	94.3±16.9 (*n*=6)
	AP amplitude	97.5±11.0 (*n*=9)	91.1±13.9 (*n*=6)	92.8±16.3 (*n*=5)	100.9±10.1 (*n*=8)	99.0±18.0 (*n*=9)	77.7±15.2* (*n*=6)
	duration (plateau phase)	91.3±23.1 (*n*=9)	76.2±20.5** (*n*=6)	91.6±14.5 (*n*=5)	95.6±19.1 (*n*=8)	78.6±13.8* (*n*=9)	77.8±16.4* (*n*=6)
	amplitude of contraction	83.5±11.7** (*n*=9)	80.5±15.9** (*n*=6)	88.8±8.1** (*n*=5)	78.6±16.8** (*n*=8)	64.2±17.9* (*n*=9)	79.7±12.6* (*n*=6)
50 μM	UEP	93.75±26.70 (*n*=4)	102.9±18.7 (*n*=5)	88.1±12.4 (*n*=6)	86.0±17.3 (*n*=8)	99.7±13.4 (*n*=5)	
	AP amplitude	81.1±22.5 (*n*=4)	89.9±19.5 (*n*=5)	93.3±17.2 (*n*=6)	97.0±3.7 (*n*=8)	88.3±19.7 (*n*=5)	
	duration (plateau phase)	101.6±13.1 (*n*=4)	80.0±25 (*n*=5)	86.0±13.3 (*n*=6)	85.2±11.7** (*n*=8)	61.7±13.9** (*n*=5)	
	amplitude of contraction	89.6±13.9 (*n*=4)	73.7±1.4** (*n*=5)	70.7±8.4^{++} (*n*=6)	80.0±19.5** (*n*=8)	63.2±18.0** (*n*=5)	
100 μM	UEP	112.0±21.5 (*n*=4)	95.0±12.3 (*n*=8)	97.9±19.4 (*n*=8)	92.8±16.1 (*n*=5)	86.9±26.7 (*n*=9)	110.0±20.6 (*n*=4)
	AP amplitude	88.6±14.0 (*n*=4)	94.0±7.5 (*n*=8)	99.2±16.1 (*n*=8)	84.5±15.9 (*n*=5)	73.0±12.3^{++} (*n*=9)	118.1±16.8 (*n*=4)
	duration (plateau phase)	117.6±32.9 (*n*=4)	73.2±10.9* (*n*=8)	76.7±12.3* (*n*=8)	90.8±15.5 (*n*=5)	42.8±8.5^{+} (*n*=9)	112.9±17.1 (*n*=4)
	amplitude of contraction	112.0±19.6 (*n*=4)	42.4±11.5^{+} (*n*=8)	67.1±12.1* (*n*=8)	69.0±19.3** (*n*=5)	43.7±10.2^{+} (*n*=9)	56.0±10.3^{+} (*n*=4)

Note. 100%: effect of the corresponding substances. $^{+}p<0.01$ and $^{++}p<0.05$ compared to bumetanide in other concentrations.

in experiments with aortic segments [7]. After pretreatment of ureteral SMC with 10 μM mesatone and histamine, bumetanide dose-dependently inhibited AP and SMC contractions (similarly to TEA, Fig. 2, *b*, *c*). BAS affects Ca^{2+} entry and activate the C-kinase pathway of Ca^{2+}-mediate regulation [5]. A direct correlation was found between phosphorylation and Na^+,K^+, $2Cl^-$ cotransport [3,6,9]. This facilitates the appearance of inhibitory effect of bumetanide on AP and contraction of SMC.

The myorelaxing effect of NP on vascular SMC is due to activation of cGMP-dependent processes [2, 6,7]. As differentiated from BAS, NP blocked Na^+,K^+, $2Cl^-$ cotransport in these cells [5,6,9]. We studied combined action of NP and bumetanide on AP and contraction of SMC in guinea pig ureter. Our previous studies showed that NP in a concentration of 100 μM stimulates contractions of ureteral SMC [2].

Pretreatment with 10 μM bumetanide facilitated the effect of NP on SMC. However, increasing the concentration of this inhibitor to 100 μM potentiated the effect of NP (Fig. 1, *d*). Bumetanide in a concentration of 10 μM abolished activation of contractions in ureteral SMC produced by NP (Fig. 2, *d*). This effect became more pronounced after increasing the concentration of this inhibitor to 100 μM (Table 2). The activating effect of NP on SMC contractions was abolished after Na^+,K^+, $2Cl^-$ cotransport blockade with bumetanide (less significantly than in experiments with BAS). The inhibitory effect of NP can be related to the blockade of the conductance of Ca^{2+}- and/or K^+-dependent chlorine channels with cGMP [14,15].

If Ca^{2+} entry is a possible target for the effect of bumetanide [7,8], it should manifest not only during activation of SMC with BAS, but also in Na^+-free solutions. Under these conditions the calcium component of AP and contractions of ureteral SMC [2,4,5] were suppressed with bumetanide in a concentration of 10 μM (Fig. 2, *e*).

Our results indicate that BAS modulate Na^+,K^+, $2Cl^-$ cotransport in ureteral SMC via activation Ca^{2+} and/or Ca^{2+}-dependent chlorine conductance of the membrane.

REFERENCES

1. D. P. Artemenko, V. A. Buryi, I. A. Vladimirova, and M. F. Shuba, *Ros. Fiziol. Zh.*, **28**, No. 3, 377-380 (1982).
2. I. V. Kovalev, A. A. Panov, Yu. L. Borodin, *et al*., *Byull. Eksp. Biol. Med*., **129**, No. 5, 539-541 (2000).
3. S. N. Orlov, S. R. Kuznetsov, I. A. Kolosova, *et al*., *Ros. Fiziol. Zh*., **83**, Nos. 5-6, 119-147 (1997).
4. S. M. Tishkin and L. V. Baidan, *Fiziol. Zh*., **28**, No. 2, 212-218 (1982).
5. M. F. Shuba and N. G. Kochemasova, *Physiology of Vascular Smooth Muscles* [in Russian], Kiev (1988).
6. N. C. Adragna, E. W. Richard, S. N. Orlov, and P. K. Lauf, *Am. J. Physiol. Cell Physiol.*, **278**, 381-390 (2000).
7. F. Akar, E. Skinner, J. D. Klein, *et al*., *Am. J. Physiol*., **276**, No. 6, 1383-1390 (1999).
8. F. Akar, G. Jiang, R. J. Paul, and W. C. O'Neill, *Am. J. Physiol. Cell Physiol.*, **281**, No. 2, 579-584 (2001).
9. C. M. Gillen and F. Bliss, *Am. J. Physiol*., **276**, No. 45, 328-336 (1999).
10. J. D. Klein and W. C. O'Neill, *Ibid*., **269**, No. 6, 1524-1531 (1995).
11. S. N. Orlov, J. Tremblay, and P. Hamet, *Ibid*., **270**, 1388-1397 (1996).
12. S. N. Orlov, J. Tremblay, and P. Hamet, *J. Membr. Biol*., **153**, No. 2, 125-135 (1996).
13. N. J. Raat, E. Delpire, C. H. van Os, and R. J. Bindels, *Pflugers Arch*., **431**, No. 3, 458-460 (1996).
14. Z. Xiong, N. Sperelakis, and R. Fenoglio-Reiser, *J. Vasc. Res*., **31**, No. 5, 271-279 (1994).
15. Y. Zhang, F. Vogalis, and R. Goyal, *Am. J. Physiol*., **274**, No. 5, 886-890 (1998).