The Effect of Neomycin on Contractile Activity of the Canine Cervical Lymphatic Vessel Induced by Various Agents

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Abstract. The effects of neomycin on isometric contractions induced by norepinephrine (NE), α_1-adrenoceptor agonist phenylephrine (PE), KCl, and an activator of GTP-binding proteins (G-proteins) NaF were studied in the isolated canine cervical lymphatic vessel (CLV). Incubation of the CLV with 0.3 or 1.3 mmol/l neomycin for 30—180 min did not affect significantly either the basal vascular tone or the response to NE, and potentiated the response to KCl by $24 \pm 6\%$ ($p < 0.05$). On the other hand, neomycin (1.3 mmol/l) treatment reduced by $22 \pm 6\%$ ($p < 0.05$) the contractions induced by PE and completely (by $96 \pm 3\%$, $p < 0.001$) suppressed the effects of NaF. Upon the combined action of NaF and NE, neomycin reduced only NaF-component of the total response. Verapamil (100 μmol/l) had no effect on the magnitude of NaF-induced tension and partially inhibited NE- and PE-induced contractions (by $20 \pm 4\%$ ($p < 0.05$) and $53 \pm 5\%$ ($p < 0.01$), respectively). Indomethacin (10 μmol/l) did not influence significantly the contractions evoked by NE, KCl, and NaF either under control conditions or in the presence of neomycin. These data suggest that the phosphoinositides may considerably contribute to the CLV contractions evoked by NaF, but do not play a substantial role in the responses of the vessel to α-adrenergic stimulation and KCl.

Key words: Lymphatic vessel — Contractile activity — Stimulants of contraction — Neomycin

Introduction

Receptor-controlled phosphodiesterase-mediated cleavage of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate and diacylglycerol is now well recognized to mediate the action of Ca-mobilizing hormones in a wide variety of systems including smooth muscles (Berridge 1987). Inositol 1,4,5-

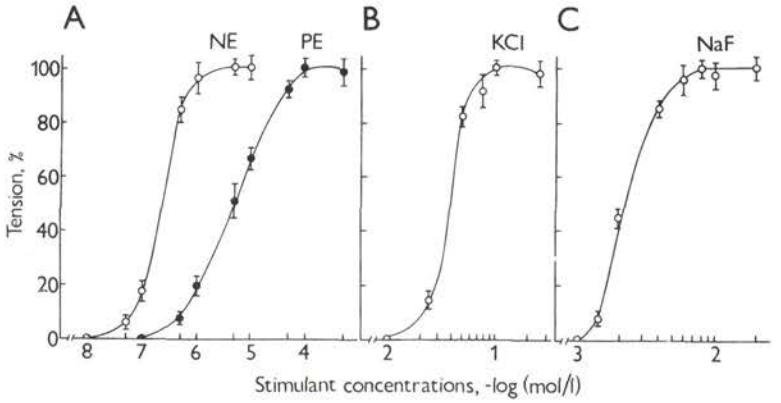

Fig. 1. Concentration-response curves; canine CLV contractile responses to: NE and PE (*A*), KCl (*B*), and NaF (*C*). *A* – NE: $EC_{50} = 0.24 \pm 0{,}02$ μmol/l ($n = 10$); PE: $EC_{50} = 5.0 \pm 0.9$ μmol/l ($n = 17$). *B* – KCl: $EC_{50} = 37.5 \pm 2.5$ mmol/l ($n = 10$). *C* – NaF: $EC_{50} = 2.2 \pm 0.2$ mmol/l ($n = 8$).

trisphosphate has been shown to produce both a transient Ca release from the sarcoplasmic reticulum (Phaneuf et al. 1987; Watras and Benevolensky 1987), and contraction of smooth muscles (Somlyo et al. 1985; Bitar et al. 1986; Kobayashi et al. 1988). Diacylglycerol is involved in the activation of protein kinase C, and also may have a role in the agonist-induced activation of smooth muscle cells (Baraban et al. 1985; Itoh and Lederis 1987). There is convincing evidence that the hormonal stimulation of phosphoinositide phosphodiesterase (phospholipase C) is mediated by GTP-binding proteins (G-proteins) (Sasaguri et al. 1986; Lo and Hughes 1987; Gaul et al. 1988).

It has been shown in many types of cells that physiological responses associated with the activation of the phosphoinositide turnover can be strongly inhibited by the aminoglycoside antibiotic neomycin (Schacht 1974; Baumann et al. 1988; Lassing and Lindberg 1988; Tysnes et al. 1988), which binds with high affinity to phosphatidylinositol 4,5-bisphosphate making the latter unavailable to phospholipase C (Williams and Schacht 1986; Reid and Gajjar 1987; Gabev et al. 1989).

The role of phosphoinositides in contractile responses has been extensively studied in smooth muscles of blood vessels (Somlyo et al. 1985; Phaneuf et al. 1987; Feltham et al. 1988; Kobayashi et al. 1988) whereas their significance for the contractility of lymphatic vessels remains unknown. The present study was designed to investigate the influence of neomycin on contractile responses of the canine cervical lymphatic vessel (CLV) induced by adrenergic agonists, KCl and/or NaF known as a stimulant of G-proteins (Cockroft 1987; Gilman 1987). The results indicate that only NaF-induced contractures are strongly blocked by neomycin.

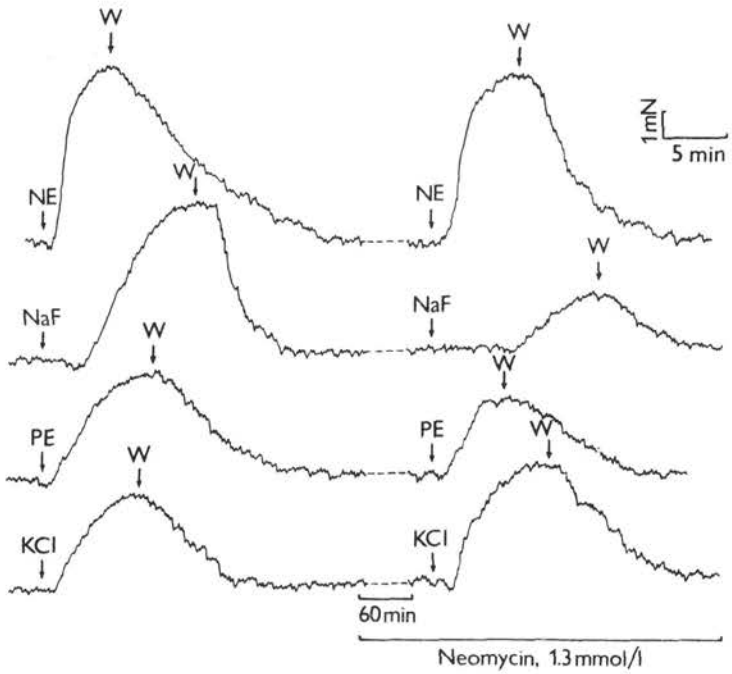

Fig. 2. Records of the contractions induced by NE (1 μmol/l), PE (100 μmol/l), NaF (10 mmol/l), and KCl (100 mmol/l) before and after the addition of 1.3 mmol/l neomycin to Krebs solution. W — washout of the stimulant.

Materials and Methods

Mongrel dogs of both sexes, weighing 10—30 kg were given i.v. anaesthesia (50 mg/kg sodium hexobarbital). The CLV was prepared and a 2—3 cm long segment containing 2—3 lymphangions was mounted on a plexiglass holder in a vertical chamber (0.5 cm^3) for isometric force measurements (Luchinin 1978). One end of the vessel was tied with silk thread to a fixed stainless steel rod, and the other end was tied to a force-displacement transducer (6 MX 1C, USSR). The preparation was superfused with a constant flow rate of 2 ml/min at 37 ± 0.1 °C by Krebs solution containing (mmol/l): Na^+ 133; K^+ 5; Ca^{2+} 2.5; Mg^{2+} 1; HCO_3^- 16.3; $H_2PO_4^-$ 1.38; Cl^- 145; glucose 7.8; pH 7.3—7.4.

Vessels were stretched to 120 % of their resting length and were allowed to equilibrate for 60 min. Experimental observations were started as soon as successive responses to NE (0.5 μmol/l), PE (10 μmol/l), NaF (10 mmol/l) or KCl (100 mmol/l), depending on the type of the experimental protocol, have turned reproducible within 5—7 %. Only one of the stimulants was used in each preparation under study except for the experiments illustrated in Fig. 4. Concentration-response curves were obtained by the single dose method. Between the stimulations (completed response — subsequent stimulation) the preparation was washed for 15 min.

Table 1. Effects of indomethacin on contractions induced by NE, NaF, and KCl in the control and after preincubation (for 60 min) with neomycin.

Conditions	Tension, %		
	NE 1 μmol/l	NaF 10 mmol/l	KCl 100 mmol/l
1. Control	100	100	100
2. Indomethacin (10 μmol/l)[a]	108 ± 5	104 ± 9	103 ± 7
3. Neomycin (1.3 mmol/l)	110 ± 7	28 ± 3**	124 ± 6*
4. Indomethacin + neomycin[b]	112 ± 6	26 ± 5**	127 ± 3*
N	5	8	8

Indomethacin was added to Krebs solution: [a] 15 min before the stimulation with NE, NaF or KCl; [b] along with neomycin throughout the incubation.
1 vs 2, and 3 vs 4 — insignificant; * 3 vs 1, and 4 vs 2 — $p < 0.05$; ** 3 vs 1, and 4 vs 2 — $p < 0.001$

Table 2. Effects of verapamil on contractions induced by NE, PE, and NaF in the control and after preincubation (for 60 min) with neomycin.

Conditions	Tension, %		
	NE 1 μmol/l	PE 100 μmol/l	NaF 10 mmol/l
1. Control	100	100	100
2. Verapamil (100 μmol/l)	80 ± 4*	47 ± 5**	100 ± 3[ns]
3. Neomycin (1.3 mmol/l)	105 ± 3[ns]	78 ± 6*	30 ± 8**
4. Verapamil + neomycin	80 ± 5*	40 ± 8*	29 ± 7[ns]
N	7	8	5

Verapamil was added to Krebs solution 15 min before the stimulation with NE, PE or NaF. 2 vs 1, 3 vs 1, and 4 vs 3: * $p < 0.05$; ** $p < 0.01$; ns — not significant. 4 vs 2: for NE and PE — not significant, for NaF — $p < 0.01$

Mean values ± SEM are given. *P* values were calculated by the Student's *t*-test. Statistical significance was assumed at $p < 0.05$.

Drugs used: L-norepinephrine bitartrate, L-phenylephrine-HCl (Serva, Heidelberg, West Germany), indomethacin, neomycin sulfate (Sigma, St. Louis, MO), verapamil (Orion, Finland).

Results

Contractile responses of the canine CLV were induced by NE, α_1-adrenoceptor agonist PE, and KCl. Figure 1 illustrates the concentration-response curves for

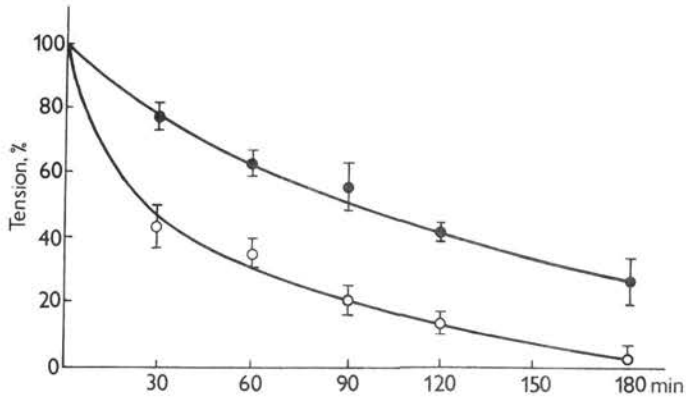

Fig. 3. Effects of 0.3 mmol/l $n = 7$ (•) and 1.3 mmol/l $n = 9$ (○) neomycin on the NaF-induced contractures. Asterisk indicates significant differences ($p < 0.01$) between 0.3 and 1.3 mmol/l neomycin.

the stimulation of CLV contractions by these agents. The sensitivity of the vessel to PE is approximately one order lower than that to NE (Fig. 1*a*). The concentrations of NE, PE and KCl producing half maximal responses (EC_{50}) were 0.24 ± 0.02 μmol/l ($n = 10$), 5.0 ± 0.9 μmol/l ($n = 7$) and 37.5 ± 2.5 mmol/l ($n = 10$), respectively. NE-induced CLV tension was of the highest amplitude, whereas the effects of PE and KCl were by 44 ± 5 % and 49 ± 7 % smaller (Fig. 2). Although the values of the CLV maximal responses to NE varied widely between individual vessels (from 2 to 5 mN), preliminary estimations ($n = 8$) indicated that this ratio of tension amplitudes evoked by PE and KCl to NE-induced contractions (approx. 1:2) was highly reproducible.

Incubation with the inhibitor of phosphoinositide metabolism, neomycin (0.3 and 1.3 mmol/l) for 30—180 min did not change the basal vascular tone and did not influence, or slightly activated the NE-induced contractile responses (107 ± 4 %, $n = 18$). The effect of PE was reduced by 22 % (78 ± 6, $n = 8$, $p < 0.05$) irrespectively of both neomycin concentration and time of incubation. Potassium-induced contractures were potentiated by 24 % in the presence of the antibiotic (124 ± 6 %, $n = 8$, $p < 0.05$) (see Figs. 2 and 4, and Tables 1 and 2).

NaF caused concentration-dependent contractures of the CLV (Figs. 1, 2). Unlike NE-, PE- and KCl-induced tension occurring with a rapid onset, responses to NaF set on after a lag-period of 3—7 min (Fig. 2) and sustained at the plateau level for at least 10—15 min (not shown). The contractures elicited by NaF (10 mmol/l) were 85 ± 5 % ($n = 8$) of the maximal contractions evoked by NE. Incubation with neomycin resulted in a significant time- and concentration-

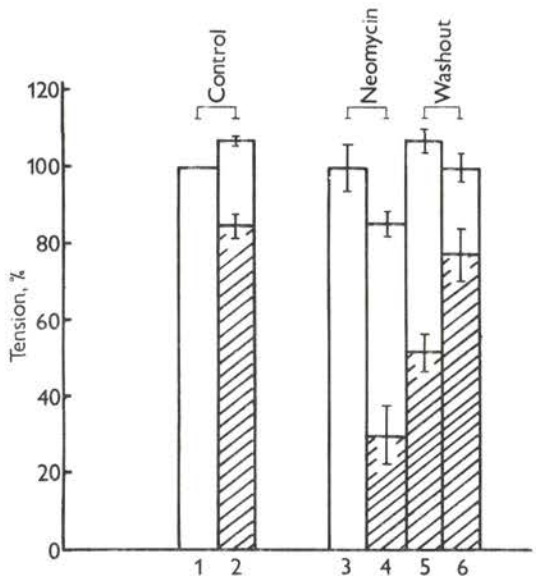

Fig. 4. The combined contractile effects of NaF (10 mmol/l) and NE (1 μmol/l) in the absence or in the presence of 1.3 mmol/l neomycin. 1 — NE-induced tension (100 %); 2 — total responses obtained upon the addition of NE to the vessels contracted by NaF; 3 and 4 — the same as in 1 and 2, respectively, but after a 60-min preincubation with neomycin; 5 and 6 — restoration of the total responses during the washout of neomycin for 120 (5) and 180 (6) min. Empty columns — NE; Hatched columns — NaF. Significant differences ($p < 0.01$; $n = 6$): * in 2 — total response vs NaF; ** NE portions — 4 and 5 vs 2; 3 vs 1 and 6 vs 2 — not significant; *** NaF portions — 4 and 5 vs 2.

dependent loss of contractile responses of the CLV accompanied by prolonging of the lag (Figs. 2 and 3).

The addition of NE (1 μmol/l) to the vessel contracted in the presence of NaF (10 mmol/l) resulted in 20 ± 2 % ($p < 0.01$) increase in the tension amplitude, which, however, did not exceed significantly the maximal response to NE (Fig. 4). In the presence of neomycin there was a decrease in the magnitude of the NaF-component and an increase in NE-component of the total response. The washout of neomycin led to the gradual restoration of the initial ratio of the contribution by each agent to the total tension reached (Fig. 4).

The inhibitor of prostaglandin synthesis, indomethacin (10 μmol/l) did not change the CLV responses to NE, KCl, and NaF either in the controls, or in the presence of neomycin (Table 1).

The Ca blocker verapamil (50—100 μmol/l) reduced the vessel responses to NE and PE by 20 and 50 %, respectively, but did not influence the NaF-induced contractures. The effects of verapamil in the presence of neomycin were similar to those in the controls (Table 2).

Discussion

The results of the present investigation showed that besides the contractile responses to known smooth muscle stimulants, α-adrenoceptor agonists and KCl, the canine CLV produces high-amplitude tension in the presence of NaF (Fig. 2), an activator of various G-proteins (Cockroft 1987; Gilman 1987). These observations are in agreement with those obtained in several types of smooth muscles (Kobayashi et al. 1988; Marc et al. 1988; Watson et al. 1988). NaF-induced contractions in the guinea-pig myometrium (Marc et al. 1988) and ileum (Watson et al. 1988) have been reported to be accompanied by the stimulation of phosphoinositide metabolism. It can be suggested that the contraction of the canine CLV produced by fluoride is also associated with the phosphoinositide response mediated by a G-protein. This suggestion was supported by our finding that the aminoglycoside antibiotic neomycin, in concentrations which have been shown to inhibit the phosphoinositide responses in intact cells and tissues (Schacht 1974; Baumann et al. 1988; Lassing and Lindberg 1988; Tysnes et al. 1988), considerably suppressed contractures of the lymphatic vessels evoked by NaF (Fig. 3).

Most probably, fluoride besides its effect on phosphoinositide turnover can also change the cAMP level in smooth muscle cells via Gs and Gi, G-proteins responsible for stimulation and inhibition of adenylate cyclase, respectively (Gilman 1987). In fact, this has been recently demonstrated by Watson et al. (1988). However, these authors found that in the presence of NaF the stimulation of cAMP production occurred much slowlier as compared with the generation of inositol phosphates in the guinea-pig ileum longitudinal muscle. They suggested that the increase in cAMP concentration could be responsible for the relatively fast (3—5 min) spontaneous relaxation of the NaF-induced contractions. cAMP has long been known to mediate vasodilatation (Bolton 1979). However, no reduction of the maximal level of tension produced in the CLV by NaF could be observed after 10—15 min. It is possible that there was no sufficient elevation of the cAMP content in the vessel muscle within this time interval.

There is strong evidence that the stimulation of α_1-adrenoceptors in various tissues induced the cell phosphoinositide response (Fleming et al. 1987; Steinberg et al. 1987; Feltham et al. 1988). The poor suppression of PE-responses and

the lack of inhibition of NE-evoked contractions by neomycin observed in this study are not consistent with the literature data, and may indicate a very small contribution of phosphoinositides to CLV contractions mediated by α-adrenergic receptors and, in particular, by their α_1-subtype.

The results of the experiments with neomycin suggest that the mechanism of the NaF-induced CLV contractions differs from that of the responses elicited by NE, PE, and KCl. This was most obvious in the experiments with combined action of NE and NaF (Fig. 4). Surprisingly, neomycin produced a small but significant potentiation of KCl-evoked tension in the CLV (Fig. 2, Table 1). To our knowledge this is the first example of a potentiating effect of the antibiotic on smooth muscle contractions. The mechanism of this effect is not clear at present. Taken together, these data suggest that of the stimulants used in our study only NaF acts essentially via the phosphoinositide turnover in the canine CLV.

The stimulation of the phosphoinositide metabolism leads to an enhanced release of arachidonic acid from diacylglycerol followed by the formation of eicosanoids (Berridge 1987). However, it is unlikely that prostaglandins are involved in the effects of NaF on the CLV or in those of NE and KCl, since indomethacin did not change the vessel responses either in the controls or in the presence of neomycin (Table 1) which itself may, at least in permeabilized human platelets, induce the release of arachidonic acid (Nakashima et al. 1987).

Likely, the mechanism underlying the development of CLV contractions in response to NaF implies preferentially intracellular Ca mobilization as the Ca antagonist verapamil, even in high concentrations which have been shown to block both voltage-operated and receptor-operated Ca-channels (Kendall et al. 1987), had no effect on NaF-induced responses (Table 2). Apparently, the influx of external Ca ions through Ca-channels is partially involved in the α-adrenergic stimulation of the lymphatic vessel, as it is seen from our data obtained with verapamil (Table 2).

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