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Facilitation of frog neuromuscular transmission by sodium fluoride

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1 The effects of sodium fluoride (NaF, 5 mM) alone or in combination with theophylline (1.5 mM) or imidazole (1.5 mM) on the amplitude of the endplate potential (e.p.p.), frequency of the miniature endplate potential (m.e.p.p.), and the quantal content of the e.p.p. of bullfrog muscle were investigated. The effects of forskolin (1 μM) and papaverine (1 μM) on the m.e.p.p. frequency were also studied.

2 NaF caused an increase of 22% in the amplitude of the e.p.p. This NaF-induced increase was enhanced by theophylline (to 51%) and reduced by imidazole (to 10%).

3 Papaverine (0.1–3 μM) increased the frequency of m.e.p.ps. Forskolin at 1 μM raised the m.e.p.p. frequency by 13%. The effect was increased to 31% by 1 μM papaverine. NaF also raised the m.e.p.p. frequency by 90%. This action too was increased by theophylline (2.6 fold) and by papaverine (2.1 fold); however, it was reduced by imidazole (1.3 fold).

4 NaF increased the quantal content of the e.p.p. by 28%. This effect was enhanced by theophylline to 44%, while it was diminished by imidazole.

5 These results suggest that an increase in the transmitter release via an elevation of cyclic AMP may be involved in the facilitation of neuromuscular transmission by NaF.

Introduction

It has been reported that adenosine 3′, 5′-cyclic monophosphate (cyclic AMP) is involved in the release of the transmitter from the motor nerve ending (Goldberg & Singer, 1969; Wilson, 1974; Dretchen *et al.*, 1976; Skirboll *et al.*, 1977; Standaert & Dretchen, 1979) and that sodium fluoride (NaF) facilitates neuromuscular transmission (Koketsu & Gerard, 1956; Jacobs & Blaber, 1971; Kaibara *et al.*, 1978; Hattori & Maehashi, 1986) by enhancing the transmitter release from the motor nerve ending (Jacobs & Blaber, 1971; Dretchen *et al.*, 1976; Standaert & Dretchen, 1979). NaF activates adenylate cyclase, thereby increasing the production of cyclic AMP from ATP. Therefore, a rise in cyclic AMP level may be related to the enhancement of the transmitter release by NaF. If so, since cyclic AMP is destroyed by phosphodiesterase, the enhancement of the transmitter release by NaF should be augmented by theophylline or papaverine (a phosphodiesterase inhibitor) and reduced by imidazole (a phosphodiesterase activator). However, this has never been confirmed electrophysiologically. In the present work we have investigated the interaction of forskolin (an adenylate cyclase activator) and papaverine on the frequency of the miniature endplate potential (m.e.p.p.). Secondly, we studied the effect of NaF (5 mM) alone and in combination with pretreatment with theophylline or imidazole on the amplitude of the endplate potential (e.p.p.), m.e.p.p. frequency, and the quantal content of the e.p.p., in order to determine whether or not an elevation of the cyclic AMP level is involved in the enhancement of transmitter release by NaF.

Methods

Sciatic nerve-sartorius muscle preparations from the bullfrog (*Rana catesbeiana*) were used. The preparation was fixed horizontally in a chamber perfused with Ringer solution. Electrical phenomena in the endplate (that is, e.p.p. and m.e.p.p.) were recorded intracellularly with a glass microelectrode filled with 3 M KCl (5–10 MΩ resistance) in the conventional way. Only those muscle cells which exhibited a resting potential of at least −80 mV were used.

To investigate the interaction of NaF and theophylline or imidazole on the e.p.p. amplitude, the e.p.p. was evoked by nerve stimulation at 0.1 Hz with a

suction electrode. Throughout this experiment, (+)-tubocurarine chloride (1.8 μg ml^{-1}) was added to the normal Ringer solution to prevent firing of the muscle fibre.

In the experiments investigating effects of forskolin and papaverine on the m.e.p.p. frequency, normal Ringer solution was used as the perfusate.

To obtain quantal contents small enough to allow application of the method of failures, transmitter release was greatly reduced by use of a modified Ringer solution in which calcium was reduced to 0.5 mM and to which 6.0 mM magnesium was added (del Castillo & Katz, 1954). Under these conditions, m.e.p.ps which appeared spontaneously and e.p.ps evoked by nerve stimulation at 1.0 Hz were simultaneously recorded, and the numbers of m.e.p.ps and e.p.ps were subsequently counted for analysis of effects of pretreatment with theophylline and imidazole. The mean quantal content was calculated by the method of failures, [$m = \log_e (N\,no^{-1})$], (m: mean quantal content, N: number of stimulations, and no: number of failures of e.p.p.)], since data obtained by this method are unaffected by alterations in receptor sensitivity, transmitter reversal potential, input resistance, or resting potential (Edelson & Nastuk, 1973).

The significance of differences between means was calculated by Student's t test. P values of 0.05 or less were considered to represent significant differences.

The composition of normal Ringer solution was as follows (mM): NaCl 110, KCl 1.9, $CaCl_2$ 1.1, NaH_2PO_4 0.5, $NaHCO_3$ 2.4 and glucose 5.6. pH was adjusted to 7.3. All experiments were performed at room temperature (20–25°C). The drugs used were 7-0-hemi-succinyl-7-deacetyl forskolin (Hoechst, West Germany) and papaverine hydrochloride, NaF, theophylline, and imidazole (Nakarai Chemicals, Japan).

Results

Effects on e.p.p. amplitude

NaF (5 mM) and theophylline (1.5 mM) tested separately had no effect on the resting potential of the muscle fibre, whereas imidazole (1.5 mM) depolarized the muscle membrane by 3–5 mV. The effects of application of NaF (5 mM) alone for 3 min and in combination with a 3 min pretreatment with theophylline (1.5 mM) or imidazole (1.5 mM) on the e.p.p. amplitude are shown in Table 1. Control values were obtained from the same cells in each experiment. Experiments to compare the effect of NaF alone with that in combination with pretreatment were not always performed on the same cell. NaF significantly increased the e.p.p. amplitude both when given alone and following pretreatment with theophylline.

Table 1 Effects of NaF (5 mM) and its interactions with theophylline (1.5 mM) and with imidazole (1.5 mM) on the e.p.p. amplitude

		e.p.p. amplitude (mV)							
Pretreatment		*None*			*Theophylline*			*Imidazole*	
Expt. No	Control	NaF	Diff	Control	NaF	Diff	Control	NaF	Diff
1	6.32	7.64	1.32	6.26	9.31	3.05	6.82	6.93	0.11
2	3.36	4.20	0.84	2.46	3.71	1.25	1.26	1.22	−0.04
3	3.36	3.75	0.39	3.21	5.65	2.44	3.59	4.28	0.69
4	4.43	5.75	1.32	2.80	4.23	1.43	2.64	3.32	0.68
5	3.47	4.14	0.67	3.74	4.83	1.09	3.86	4.26	0.40
6	1.90	2.05	0.15						
7	4.72	5.72	1.00						
8	3.62	5.00	1.38						
9	2.08	2.60	0.52						
10	4.52	5.52	1.00						
Mean ± s.e.mean of Diff		0.86 ± 0.13****				1.85 ± 0.38**,**			0.37 ± 0.15*

Diff: difference (NaF – Control); s.e.mean: standard error of mean.
Differences statistically significant as compared with the control value at $^{**}P < 0.01$ and $^{****}P < 0.001$ (two sided paired t test).
Significantly different from the value (difference) obtained in the case of application of NaF alone at $^{*}P < 0.05$ and $^{**}P < 0.01$ (two sided simple t test).

Table 2 Effects of papaverine (0.1–3 μM) on the m.e.p.p. frequency

	m.e.p.p. frequency (min^{-1})											
Papaverine (μM)	0.1			0.3			1			3		
Expt. No	Control	Pap	Diff	Control	Pap	Diff	Control	Pap	Diff	Control	Pap	Diff
1	40.0	47.3	7.3	12.0	12.0	0.0	44.7	52.3	7.6	28.7	46.3	17.6
2	32.7	32.3	−0.4	6.3	7.0	0.7	56.7	67.3	10.6	27.0	49.7	22.7
3	29.7	25.7	−4.0	40.3	47.3	7.0	107.7	132.0	24.3	13.7	23.7	10.0
4	25.0	24.3	−0.7	128.7	129.3	1.0	126.3	157.0	30.7	25.3	35.3	10.0
5	23.0	23.3	0.3	32.7	36.0	3.3	84.0	118.3	34.3	15.7	22.3	6.6
Mean ± s.e.mean of Diff			0.5 ± 1.9			2.4 ± 1.3			21.5 ± 5.3*			13.4 ± 2.9*

Pap: papaverine; Diff: difference (Pap − control).
Difference statistically significant as compared with the control value at $*P < 0.05$ (two sided paired t test).

Table 3 Effects of forskolin (1 μM) and its interaction with papaverine (1 μM) on the m.e.p.p. frequency

	m.e.p.p. frequency (min^{-1})					
Pretreatment	*None*			*Papaverine*		
Experiment No	Control	Forskolin	Difference	Control	Forskolin	Difference
1	118.3	128.3	10.0	46.0	61.2	15.2
2	106.5	122.5	16.0	105.7	136.4	30.7
3	248.6	287.4	38.8	172.5	227.7	55.2
4	263.8	281.6	17.8	103.4	138.8	35.4
5	227.9	247.3	19.4	181.9	220.5	38.6
6	144.7	162.0	17.3	67.3	90.8	23.5
7	155.4	196.7	41.3	53.2	71.8	18.6
Mean ± s.e.mean of Difference			22.9 ± 4.6***			31.0 ± 5.2****

Difference: Forskolin − control.
Difference statistically significant as compared with the control value at $***P < 0.005$ and $****P < 0.001$ (two sided paired t test).

Moreover, there was a significant difference between the values obtained in both cases; that is, theophylline significantly enhanced the increase in e.p.p. amplitude caused by NaF. On the other hand, the pretreatment with imidazole reduced it and nullified any significant difference between the values of the control and NaF-treated cells.

Effects on m.e.p.p. frequency

Papaverine (0.1–3 μM) raised the m.e.p.p. frequency dose-dependently as shown in Table 2. The effects of forskolin (1 μM) alone and its interaction with papaverine (1 μM) pretreatment on the m.e.p.p. frequency are shown in Table 3. Forskolin significantly raised the m.e.p.p. frequency. Its increase by forskolin was augmented by papaverine although it should be noted that the control values in the experiment for papaverine pretreatment were, by chance, significantly ($P < 0.05$) smaller than those in the case of no pretreatment. Hence it is the difference between the normalised values that is significant.

The effects of NaF (5 mM) alone and in combination with theophylline (1.5 mM), papaverine (1 μM) or imidazole (1.5 mM) on the m.e.p.p. frequency are shown in Table 4. In this table, the m.e.p.p. frequency has been expressed logarithmically because the differences between the variances of raw control values in the experiment without pretreatment and in the case of pretreatment with theophylline or papaverine were so significant ($P < 0.05$) that it was impossible to make a comparison between the effects of NaF without pretreatment and following pretreatment with theophylline or papaverine. The results of this experiment are illustrated in part in Figure 1. NaF significantly raised the m.e.p.p. frequency. Theophylline and papaverine moreover enhanced its rise by NaF. On the contrary, imidazole may have reduced the rise in m.e.p.p. frequency effected by NaF. Thus, there were significant differences between the effects seen with NaF alone and when fluoride was given after pretreatment with theophylline or imidazole. These results show that NaF increased transmitter release and that theophylline and papaverine were synergistic with NaF, while imidazole was antagonistic.

Effects on the quantal content of the e.p.p.

The effects of NaF (5 mM) alone and its interaction with theophylline (1.5 mM) or imidazole (1.5 mM) on the quantal content of the e.p.p. are shown in Table 5. NaF significantly increased the quantal content. Theophylline enhanced this NaF-induced increase. However, imidazole diminished the effect of NaF. These observations suggest that cyclic AMP may be involved in the facilitation of transmitter release by NaF.

Table 4 Effects of NaF (5 mM) and its interactions with theophylline (1.5 mM), papaverine (1 μM) and with imidazole (1.5 mM) on the m.e.p.p. frequency

Pretreatment	*None*			log_{10}(*m.e.p.p.s min*$^{-1}$) *Theophylline*			*Papaverine*			*Imidazole*		
Expt. No	Control	NaF	Diff	Control	NaF	Diff	Control	NaF	Diff	Control	NaF	Diff
1	1.33	1.69	0.36	0.83	1.24	0.41	2.02	2.36	0.34	2.26	2.34	0.08
2	1.75	2.05	0.30	1.69	2.15	0.46	1.87	2.21	0.34	2.89	3.05	0.16
3	2.42	2.64	0.22	1.95	2.32	0.37	1.68	2.00	0.32	1.18	1.28	0.10
4	2.85	3.07	0.22				2.09	2.38	0.29			
5	2.05	2.30	0.25				1.94	2.27	0.33			
6	1.19	1.49	0.30									
Mean ± s.e.mean of Diff		0.28 ± 0.02****			0.41 ± 0.03***.**			0.32 ± 0.01****			0.11 ± 0.02*.***	

Values listed in this table represent the logarithm of the frequency. Diff: difference (NaF − control).
Significantly different from the control value at $*P < 0.05$, $***P < 0.005$ and $****P < 0.001$ (two sided paired t test).
Significantly different from the value (difference) obtained in the case of application of NaF alone at $**P < 0.01$ and $***P < 0.005$, respectively (two sided simple t test).

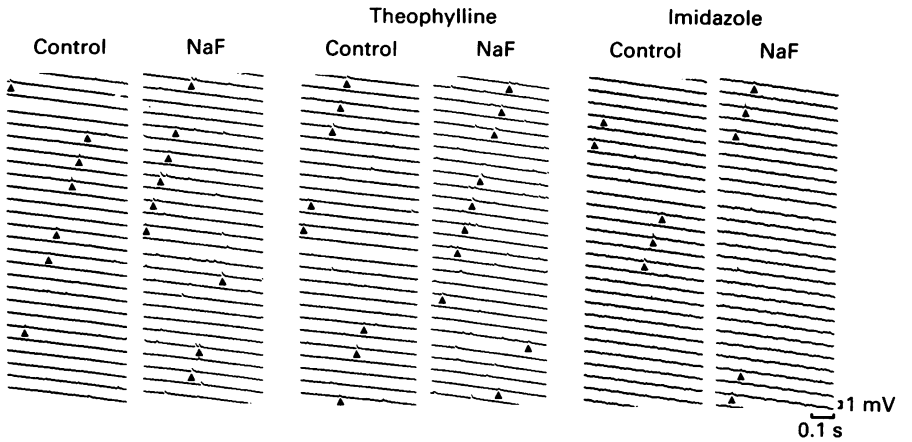

Figure 1 Interactions between NaF (5 mM) and theophylline (1.5 mM) or imidazole (1.5 mM) on the e.p.p. and m.e.p.p. that appeared in the sartorius muscle endplate which had been treated with 0.5 mMCa^{2+} and 6.0 mMMg^{2+}. NaF significantly raised the m.e.p.p. frequency. This rise was enhanced by pretreatment of the muscle with theophylline for 3 min, while it was diminished by imidazole pretreatment. (▲) e.p.p. which is evoked by sciatic nerve stimulation at 1 Hz. NaF decreased the number of failures of e.p.p.

Discussion

Several investigators (Koketsu & Gerard, 1956; Jacobs & Blaber, 1971; Dretchen *et al.*, 1976; Skirboll *et al.*, 1977; Kaibara *et al.*, 1978; Standaert & Dretchen, 1979; Hattori & Maehashi, 1986) have found that NaF facilitates neuromuscular transmission. There are two major sites of action of NaF which are

Table 5 Effects of NaF (5 mM) and its interactions with theophylline (1.5 mM) and with imidazole (1.5 mM) on the quantal content of the e.p.p.

	Quantal content of the e.p.p.						
	Control			*NaF*			*Difference*
Experiment No	N	no	m_1	N	no	m_2	m_2-m_1
1	175	124	0.34	178	109	0.49	0.15
2	177	104	0.53	175	97	0.59	0.06
3	180	101	0.58	178	87	0.72	0.14
4	164	102	0.47	164	82	0.69	0.22
5	168	134	0.23	163	124	0.27	0.04
6	173	147	0.16	178	146	0.20	0.04
			Mean ± s.e.mean of (m_2-m_1):				0.11 ± 0.03*
			Theophylline pretreatment				
7	175	100	0.56	174	69	0.92	0.36
8	170	91	0.62	174	78	0.80	0.18
9	178	126	0.35	173	106	0.49	0.14
			Mean ± s.e.mean of (m_2-m_1):				0.23 ± 0.07
			Imidazole pretreatment				
10	154	132	0.15	164	143	0.14	−0.01
11	176	142	0.21	174	141	0.21	0.00
12	170	113	0.41	172	111	0.44	0.03
			Mean ± s.e.mean of (m_2-m_1):				0.01 ± 0.01

N: number of stimulations; no: number of failures of e.p.p.; m_1 and m_2: mean quantal content obtained before and after NaF application, respectively.
Significantly different from the control value (m_1) at $^*P < 0.05$ (two sided paired t test).

described in these papers, the presynaptic (Jacobs & Blaber, 1971; Dretchen *et al.*, 1976; Skirboll *et al.*, 1977; Standaert & Dretchen, 1979) and postsynaptic membrane (Kahlson & Uvnäs, 1938; Koketsu & Gerard, 1956; Kaibara *et al.*, 1978). Koketsu & Gerard (1956) and Kaibara *et al.* (1978) found that NaF increased the e.p.p. amplitude, but not the m.e.p.p. frequency nor the quantal content of e.p.p. On the other hand, Jacobs & Blaber (1971) have reported that NaF increases the e.p.p. amplitude and its quantal content by increasing the mobilization rate of the transmitter. In the present study, we too investigated the effect of NaF on the presynaptic membrane. NaF significantly increased the e.p.p. amplitude, m.e.p.p. frequency, and the quantal content of the e.p.p. This indicates that NaF acted on the presynaptic membrane and enhanced transmitter release, since m.e.p.p. frequency is dependent on the properties of the presynaptic membrane (Katz, 1962) and the increase of the quantal content means the facilitation of the release evoked by nerve stimulation (del Castillo & Katz, 1954). This notion underlies the description of Jacobs & Blaber (1971).

Effects of theophylline on neuromuscular transmission have often been investigated to determine whether cyclic AMP is related to transmitter release from the motor nerve ending (Goldberg & Singer, 1969; Wilson, 1974; Skirboll *et al.*, 1977; Standaert & Dretchen, 1979). Goldberg & Singer (1969) have shown that theophylline increases the e.p.p. amplitude, m.e.p.p. frequency, and the quantal content of the e.p.p. Wilson (1974) has observed that theophylline increases the e.p.p. amplitude and its quantal content. Moreover, Standaert & Dretchen (1979) have reported that the augmentation of the muscle twitch by NaF is enhanced by pretreatment with theophylline, while it is diminished by imidazole.

In our work forskolin raised the m.e.p.p. frequency. Papaverine not only raised the m.e.p.p. frequency itself, but also augmented its rise by forskolin. This implies that there is a basal activity of cyclic AMP in controlling the transmitter release from the presynaptic terminal.

Since NaF significantly increased the e.p.p. amplitude, m.e.p.p. frequency, and the quantal content, it was suggested that the increase in the transmitter release is related to facilitation of neuromuscular transmission by NaF. These augmentative actions of NaF were all enhanced by theophylline while they were diminished by imidazole. In addition, the rise in m.e.p.p. frequency induced by NaF was potentiated by papaverine. These findings lead us to the conclusion that a rise in the cyclic AMP level is indeed involved in the increase of transmitter release by NaF and that this action contributes to the facilitation of neuromuscular transmission by NaF. This conclusion supports the interpretations previously mentioned by Dretchen *et al.* (1976), Skirboll *et al.* (1977), and Standaert & Dretchen (1979).

Most laboratories doing similar experiments use theophylline (a xanthine derivative) on the assumption that observed effects are due to inhibition of phosphodiesterase, even though theophylline has other actions (Standaert & Dretchen, 1981) e.g., induction of the calcium release from its storage site in the cytoplasm (Bianchi, 1961). To deal with the possibility that another action might be responsible for the increase in the transmitter release, we used papaverine (a non-xanthine derivative), which shares theophylline's capacity to inhibit phosphodiesterase but not its other characteristics (Standaert & Dretchen, 1981). Papaverine acted like theophylline on the transmitter release, that is, papaverine enhanced the rise in m.e.p.p. frequency by forskolin and by NaF. This suggests that the actions of theophylline and papaverine are due to the rise in the cyclic AMP level.

As depolarization of the membrane decreases the e.p.p. amplitude (Takeuchi & Takeuchi, 1959), the decrease in the resting potential by imidazole might be related to the decrease in e.p.p. amplitude. However, it is unquestionable that imidazole acts on the nerve ending since imidazole caused a reduction in the increase in m.e.p.p. frequency and in the quantal content produced by NaF.

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