[Original] Matsumoto Shigaku 24 : 46~51, 1998

key words : stannic chloride — tartaric acid — neuromuscular transmission — mouse

Inhibition of Mouse Neuromuscular Transmission by Stannic Chloride

TOSHIMI HATTORI and HIROSHI MAEHASHI

Department of Dental Pharmacology, Matsumoto Dental University School of Dentistry
(Chief: Prof. H. Maehashi)

Summary

The present study was conducted to determine whether stannic chloride ($SnCl_4$) at concentrations above 50 μM inhibits or facilitates neuromuscular transmission. $SnCl_4$ decreased the amplitude of the endplate potential (e.p.p.) concentration-dependently in a concentration range from 0.05 to 0.5 mM. Not only $SnCl_4$ (0.5 mM) but also tartaric acid (TA, 0.5 mM), a solvent for 0.5 mM $SnCl_4$, decreased the e.p.p.. The action of $SnCl_4$ was stronger than that of TA. Both $SnCl_4$ (0.5 mM) and TA (0.5 mM) decreased the quantal content of the e.p.p., and the action of the former was significantly stronger than that of the latter. $SnCl_4$ (0.5 mM) raised the frequency of the miniature endplate potential (m.e.p.p.), whereas TA (0.5 mM) reduced it. $SnCl_4$ (0.5 mM) decreased the m.e.p.p. amplitude as did TA (0.5 mM). The results obtained show that $SnCl_4$ inhibits neuromuscular transmission by decreasing the volume of the transmitters released by nerve impulses.

Introduction

It has been reported that stannous fluoride (SnF_2) is significantly more effective than sodium fluoride (NaF) in the reduction of the incidence of dental caries in schoolchildren[1]. SnF_2 is used as a prophylactic containing tin for dental caries, but stannic fluoride (SnF_4) has not been used for the same purpose. These facts suggest that not only F^- but also Sn^{2+} have a prophylactic effect on dental caries and that Sn^{2+}, a divalent tin ion but not a tetravalent one, is able to prevent dental caries.

In our previous experiment of comparing the effects of $SnCl_2$ with those of $SnCl_4$, $SnCl_2$ at 30 μM facilitated neuromuscular transmission, but $SnCl_4$ at the same concentration as that of $SnCl_2$ had no effect[2]. If $SnCl_4$ tends to inhibit neuromuscular transmission, it follows that the effects of these tin compounds on dental caries correspond with those on the neuromuscular transmission, and moreover, it appears likely that these properties of $SnCl_2$ are related to both the prevention of dental caries and the facilitation of neuromuscular transmission.

The present study was conducted to determine whether $SnCl_4$, at comparatively high concentrations above 50 μM, inhibits or facilitates neuromuscular transmission and to confirm that the effects of Sn^{2+} and Sn^{4+} on neuromuscular transmission correspond with those on the prevention of dental caries. The

(submitted January 26, 1998 ; accepted March 12, 1998)

results obtained suggest that $SnCl_4$ has an inhibitory action on neuromuscular transmission.

Materials and Methods

Phrenic nerve-diaphragm preparations from male ICR mice (body weight, 40–50 g) were used as the material. The material was horizontally mounted in a chamber and perfused with saline composed of (in mM) NaCl, 154 ; KCl, 5 ; $CaCl_2$, 2 ; $MgCl_2$, 1 ; glucose, 11 ; and HEPES, 5[9]. The pH was adjusted to 7. 3. The perfusate was aerated with a mixture gas (95% O_2 + 5% CO_2) throughout the experiment. The endplate potential (e.p.p.) and the miniature endplate potential (m.e.p.p.) at the neuromuscular junction were recorded by conventional intracellular recording method with glass microelectrodes. The quantal content of the e.p.p. was calculated by the method of failures, i. e., $m=\log_e(N/n)$ (m : mean quantal content, N : number of stimulations, n : number of failures of e.p.p.)[9]. To record the e.p.p., *d*-tubocurarine at a concentration of 1.0 μM was added to the perfusate. As a solvent for $SnCl_4$, an aqueous solution of tartaric acid (TA) at the same concentration as that of $SnCl_4$ was used to prevent $SnCl_4$ from precipitating to form an insoluble salt[5,6].

The chemicals used in this study were $SnCl_4$, TA, and *d*-tubocurarine chloride, all obtained from Nacalai Tesque (Japan). Each value of the data represents the mean value ± the standard error of the mean and the number of experiments (N). Statistical analyses of the data were performed by the Student's 2-sided paired *t*-test if not mentioned. Differences between mean values were considered significant if the probability of error (p) was less than 0.05.

Results

Fig.1 illustrates the concentration-response relationship of $SnCl_4$ on the e.p.p. amplitude in the concentration range from 0.05 to 0.5 mM. $SnCl_4$ decreased the e.p.p. concentration-dependently. $SnCl_4$ at concentrations above 0.1 mM significantly decreased the e.p.p.. $SnCl_4$ (0.5 mM) decreased the e.p.p. to less than a half of the control value (control, 3.00 ± 0.41 mV ; $SnCl_4$, 1.37 ± 0.29 mV ; N=10).

To elucidate the detailed mode of inhibitory actions of $SnCl_4$, Not only the effects of $SnCl_4$ (0.5 mM) but also those of TA (0.5 mM), a solvent for 0.5 mM $SnCl_4$, were investigated on the electric phenomena at the neuromuscular junctions. Fig. 2A illustrates the wave forms of the e.p.p. observed before and after applications of $SnCl_4$ and TA. Fig.2B illustrates the statistical analyses of the data obtained. As shown in these figures, both $SnCl_4$ and TA significantly decreased the e.p.p., but the extent of the decrease induced by $SnCl_4$ was larger than that of the decrease induced by TA. The mean value of the difference between $SnCl_4$ and its control was significantly different from that of the difference between TA and its control at $p<0.001$ by simple *t*-test. In addition, there was no significant difference between both the control values for $SnCl_4$ and TA.

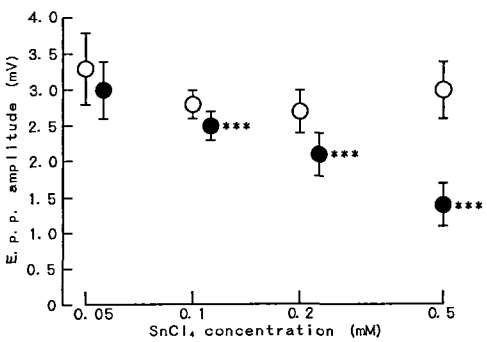

Fig.1 : Concentration-response relationship of $SnCl_4$ on the e. p. p. amplitude. $SnCl_4$ decreased the e. p. p. concentration-dependently in the concentration range from 0.05–0.5 mM. ○ and ●: E. p. p. amplitude before and after $SnCl_4$ application. ***: $p<0.005$. N=10 (in every experiment).

To ascertain how $SnCl_4$ decreased the e.p.p., the actions of $SnCl_4$ and TA on the quantal content of the e.p.p. were examined. As shown in Fig.

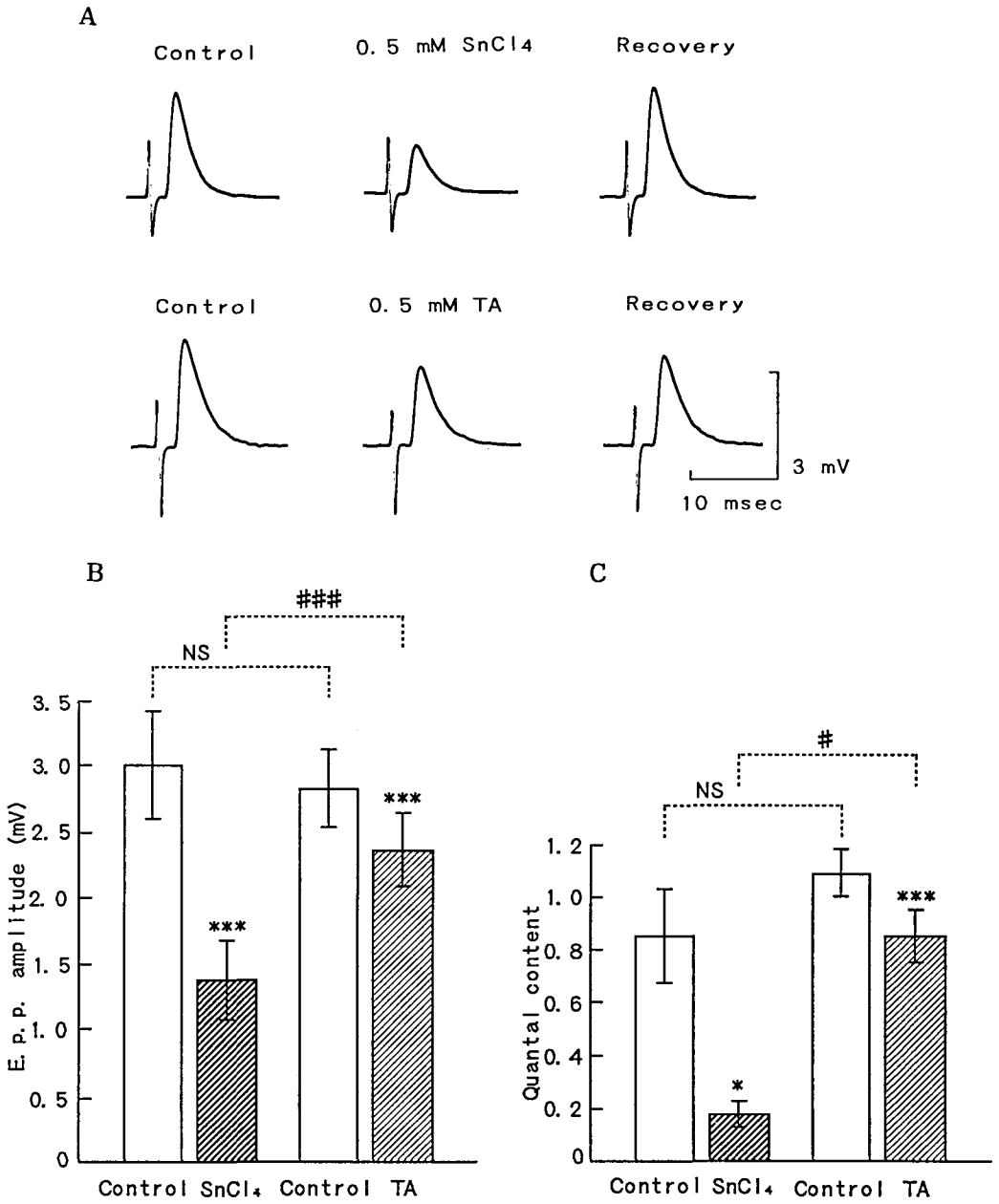

Fig. 2 : Effects of $SnCl_4$ and TA on the e.p.p. and the quantal content. (A) Wave forms of the e.p.p. observed immediately before and 2 min after application of $SnCl_4$ and TA and 5 min after withdrawal of them. (B) Statistical analyses of the data on the e.p.p. amplitude. N=10 ($SnCl_4$) and 12 (TA). The action of $SnCl_4$ (0.5 mM) was significantly stronger than that of TA (0.5 mM). (C) Statistical analyses of the data on the quantal content of the e.p.p.. N=6 ($SnCl_4$) and 12 (TA). $SnCl_4$ (0.5 mM) significantly decreased the quantal content more powerfully than TA (0.5 mM). * and *** : $p<0.05$ and 0.005, respectively. NS, # and ### : No significance, $p<0.05$ and 0.005 by simple t-test.

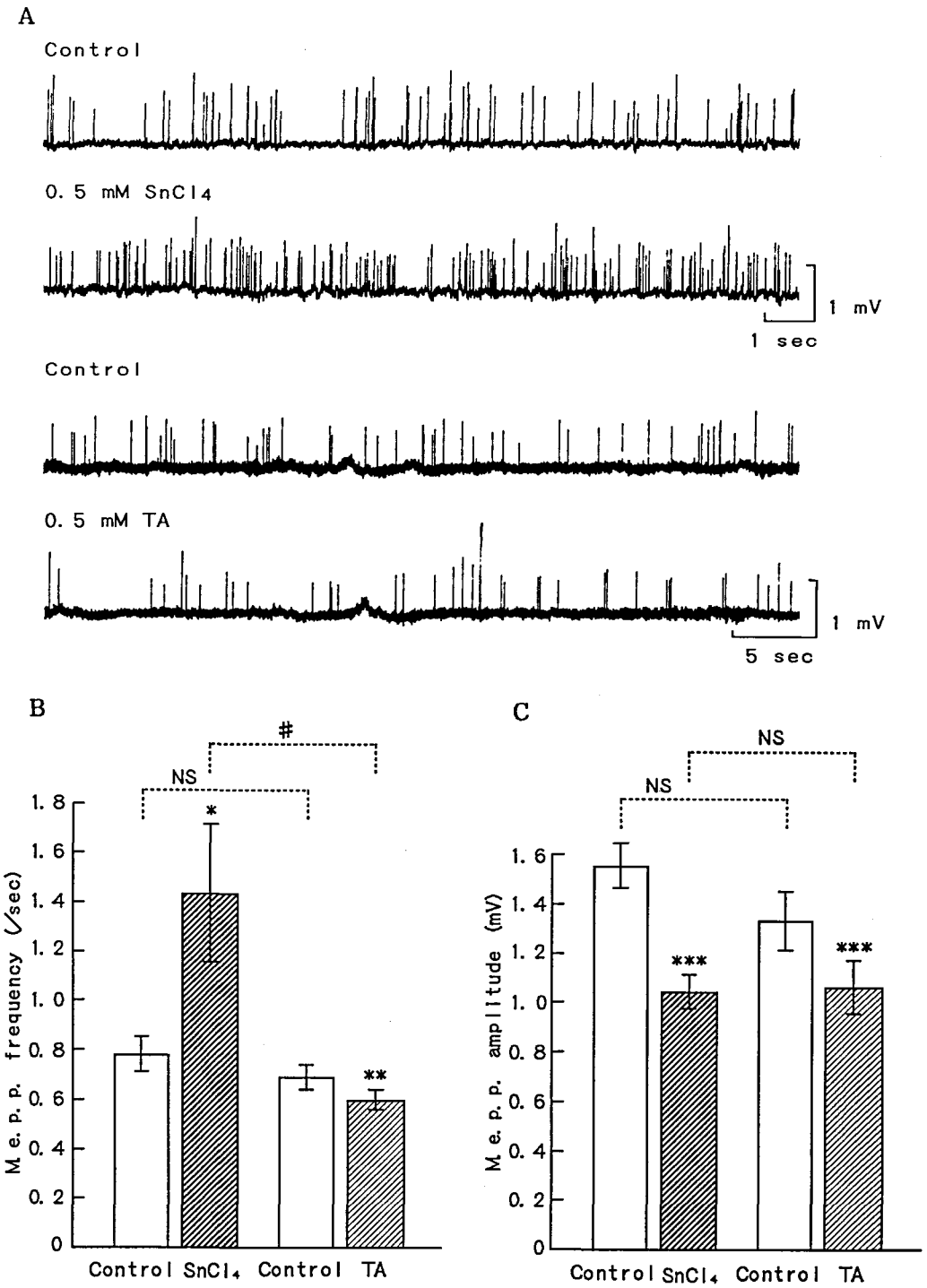

Fig. 3 : Effects of $SnCl_4$ and TA on the m.e.p.p.. (A) Traces of the m.e.p.p. recorded immediately before and 2 min after applications of $SnCl_4$ and TA. (B) Statistical analyses of the data on the m.e.p.p. frequency. N =12 ($SnCl_4$) and 15 (TA). $SnCl_4$ (0.5 mM) raised the m.e.p.p. frequency, but TA (0.5 mM) reduced it. (C) Statistical analyses of the data on the m.e.p.p. amplitude. N= 12 ($SnCl_4$) and 15 (TA). *, ** and ***: $p<0.05$, 0.01 and 0.005, respectively. NS and # : No significance and $p<0.05$ by simple *t*-test.

2C, both $SnCl_4$ (0.5 mM) and TA (0.5 mM) decreased the quantal content. The action of the former was much stronger than that of the latter. The mean value of the difference between $SnCl_4$ and its control was significantly different from that of the difference between TA and its control at $p< 0.001$ by simple *t*–test.

To compare the influences of $SnCl_4$ and those of TA on spontaneous transmitter release and on acetylcholine (ACh) sensitivity of the muscle endplate, the effects of $SnCl_4$ and TA on the m.e.p.p. were investigated. As shown in Fig. 3A, $SnCl_4$ (0.5 mM) raised the m.e.p.p. frequency whereas TA (0.5 mM) reduced it. Fig. 3B illustrates the statistical analyses of the data. The mean value of the difference between $SnCl_4$ and its control was significantly different from that of the difference between TA and its control at $p< 0.001$ by simple *t*–test. As shown in Fig. 3C, both $SnCl_4$ and TA decreased the m.e.p.p. amplitude. However, the mean value of the difference between $SnCl_4$ and its control was not significantly different from that of the difference between TA and its control by simple *t*–test.

Discussion

Brûlé *et al*.[6] reported that $SnCl_4$ (0.11 mM) has inhibitory effects on the electrical and mechanical activities of crab skeletal muscle. That is, $SnCl_4$ decreases the resting potential, height of the action potential, and the twitch tension. However, the effects of $SnCl_4$ on neuromuscular transmission has not yet been investigated.

The electrical response of the neuromuscular junction observed in our experiment, that is, the decrease in the e.p.p. amplitude induced by $SnCl_4$ suggests that $SnCl_4$ either inhibits transmitter release or reduces the ACh sensitivity of the endplate. The result of $SnCl_4$ decreasing the quantal content shows that $SnCl_4$ inhibits the evoked release of the transmitter. Furthermore, the m.e.p.p. amplitude was decreased not only by $SnCl_4$ but also by TA, indicating that reduction in the ACh sensitivity of the endplate is due to TA but not to $SnCl_4$. The $SnCl_4$–induced rise in m.e.p.p. frequency shows that $SnCl_4$ increases spontaneous transmitter release. In summary, these results show that $SnCl_4$ decreases the evoked release of the transmitter but on the other hand, increases its spontaneous release.

Kita *et al*.[7] reported that increasing the concentration of Ni^{2+}, Mn^{2+}, or Co^{2+} in the extracellular solution increases the m.e.p.p. frequency in the absence of Ca^{2+}, but in the presence of Ca^{2+}, reduces it and decreases the e.p.p. amplitude. The properties of Sn^{4+} resemble those of these ions but are not exactly the same as them as $SnCl_4$ raised the m.e.p.p. frequency even in the presence of Ca^{2+} (2 mM, see Methods). From this, it can be said that Sn^{4+} is a unique metal ion different from the ions mentioned above. Moreover, it is suggested that there is a difference in mechanism between an evoked transmitter release and a spontaneous one.

In conclusion, the findings obtained show that $SnCl_4$ inhibits neuromuscular transmission by decreasing the volume of the transmitter released by nerve impulses and furthermore, show that the effects of Sn^{2+} and Sn^{4+} on the evoked transmitter release correspond with those on the prevention of dental caries.

Additional experiments are needed to elucidate the reason why the effect of $SnCl_4$ on an evoked transmitter release is different from that on a spontaneous release. Due to the facts that Ca^{2+} is indispensable for both the calcification of teeth and transmitter release and that $SnCl_2$ increases Ca^{2+} influx into the nerve terminal[8], it is speculated that the common property of $SnCl_2$ which is involved in both the prevention of dental caries and the facilitation of transmitter release might be an accelerating action on the Ca^{2+} uptake into the cells in each of the tissues.

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抄録：塩化第二スズによるマウス神経筋伝達の抑制

服部敏己，前橋　浩（松本歯大・歯科薬理）

フッ化第一スズは齲蝕予防薬として使われ，それはフッ化ナトリウムよりも齲蝕予防効果が強いとの報告があるが，フッ化第二スズについてはそのような報告はない．これらのことからフッ化第一スズの齲蝕予防効果には第一スズイオンが関与していることが推察される．これまでの実験から塩化第一スズ（$SnCl_2$）は運動神経末端においてCa^{2+}流入量を増大させることにより神経筋伝達を促進することが明らかにされており，2価および4価のスズ化合物の神経筋伝達への作用と齲蝕予防効果とが対応しているように思われる．ここではそのことを確かめるために塩化第二スズ（$SnCl_4$）が神経筋伝達を促進するのかあるいは抑制するのかその作用について調べた．

材料にはICR系雄性マウス（体重：40–50 g）の横隔膜神経筋標本を用いた．材料をchamber内に水平に固定して灌流した．3 M KClを充填したガラス微小電極により終板電位（e.p.p.）および微小終板電位（m.e.p.p.）を細胞内誘導した．e.p.p.記録に際しては1 μM *d*-tubocurarineを灌流液に添加した．e.p.p.のquantal contentの測定にはmethod of failuresを用いた．$SnCl_4$はそれと同濃度の酒石酸水溶液に溶解して適用した．

その結果$SnCl_4$（0.05–0.5 mM）はe.p.p.振幅を濃度依存的に減少させた．$SnCl_4$（0.5 mM）の溶媒の酒石酸（TA，0.5 mM）もe.p.p.を有意に抑制したが，$SnCl_4$によるそれより弱かった．e.p.p.のquantal contentに対しても$SnCl_4$はTAよりも強く抑制した．m.e.p.p.に対しては$SnCl_4$は発生頻度を上げ，TAは下げた．m.e.p.p.振幅に対して$SnCl_4$もTAも減少させたが両者間に有意差はなかった．以上の結果から$SnCl_4$は神経衝撃により遊離される伝達物質の量を減少させることにより神経筋伝達を抑制すること，そしてこのことが$SnCl_4$が齲蝕予防薬として使われないことと関係している可能性が示唆された．