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Comparison between Effects of Stannous Chloride and Stannic Chloride on the Transmitter Release from Mouse Motor Nerve Terminals

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Summary

Stannous chloride ($SnCl_2$) is thought to facilitate the transmitter release from the motor nerve terminals. Stannous ions tend to become oxidized to stannic ions (Sn^{4+}) in the presence of water and oxygen. Therefore, the results seen with $SnCl_2$ until now might reflect the properties of Sn^{4+}. In the present study, to ascertain whether $SnCl_2$ facilitates the transmitter release from mouse motor nerve terminals, we compared the effects of stannic chloride ($SnCl_4$) on the miniature endplate potential (m. e. p. p.) frequency and the quantal content of the endplate potential (e. p. p.) with those of $SnCl_2$. We statistically analyzed the mean values of the differences of $SnCl_2$ (30 μM) from its control and of $SnCl_4$ (30 μM) from its control. There were significant differences between the two, both in the m. e. p. p. frequency and in the quantal content. The results obtained demonstrate that the actions of Sn^{4+} are not involved in the effects shown by $SnCl_2$. We conclude that $SnCl_2$ facilitates the transmitter release from mouse motor nerve terminals.

Introduction

Stannous fluoride, a prophylactic against dental caries, augments the twitch of the skeletal muscle[1]. In experiments examining the effects of stannous chloride ($SnCl_2$), we have clarified that this effect involves not only the properties of fluoride ions but also those of stannous ions (Sn^{2+})[1]. That is, it is suggested that $SnCl_2$ facilitates the transmitter release from the motor nerve terminals[2].

Because Sn^{2+} has a tendency to be oxidized to stannic ion (Sn^{4+}) in the presence of water and oxygen[3], the results seen with $SnCl_2$ until now perhaps might involve the actions of Sn^{4+}, and it has not been established that all of the actions of $SnCl_2$ are ascribed to $SnCl_2$ itself. In the present study, with the objective of ascertaining whether $SnCl_2$ facilitates the transmitter release, we compared the effects of stannic chloride ($SnCl_4$) on the electrical activities of the muscle endplate with those of $SnCl_2$.

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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 the chamber and perfused with the saline composed of (in mM) : NaCl, 154 ; KCl, 5 ; $CaCl_2$, 2 ; $MgCl_2$, 1 ; glucose, 11 ; and HEPES, 5[4]. The pH was adjusted to 7.3. The perfusate was aerated with a gas mixture (95% O_2+5% CO_2) throughout the experiment. The endplate potential (e. p. p.) and the miniature endplate potential (m. e. p. p.) were recorded by a conventional intracellular recording method with glass microelectrodes. *d*-Tubocurarine (1.0 μM) was added to the perfusate to record the e. p. p.. The quantal content of the e. p. p. was calculated by the method of failures, i. e., $m=\log_e(N/n)$ in which m is the mean quantal content, N the number of stimulations, and n the number of failures of e. p. p.[5]. The isotonicity of the high potassium (K)-medium containing KCl 5-fold the normal content was maintained by reduction of the NaCl concentration. An aqueous solution of 10 mM tartaric acid (TA) was used as a solvent for $SnCl_2$, yielding 4 mM $SnCl_2$ immediately before the experiment[3]. The final concentration of $SnCl_2$ and of TA in the perfusate was 30 μM and 75 μM, respectively. They significantly facilitate the transmitter release in the mouse[6]. When the effects of 30 μM $SnCl_4$ were examined, 75 μM TA was added to the perfusate to make it easier to compare the effects of $SnCl_4$ with those of $SnCl_2$.

Chemicals used in this study ($SnCl_2$, $SnCl_4$, TA, and *d*-tubocurarine chloride) were obtained from Nacalai Tesque (Japan). Each value presented in the results represents the mean value± standard error of the mean for the number of experiments (N) indicated. Statistical analyzes of the data were performed using the Student's 2-tailed paired *t*-test if not mentioned. Differences between mean values were considered significant when the probability of occurrence by chance (p) was less than 0.05.

Results

Initially, to clarify the effects of $SnCl_2$ and $SnCl_4$ on the spontaneous transmitter release, we examined their actions on the m. e. p. p. frequency in the high K-medium. Figure 1 illustrates the comparison between the effects of $SnCl_2$ and $SnCl_4$. $SnCl_2$ (30 μM) significantly increased the m. e. p. p. frequency, while $SnCl_4$ (30 μM) had no effect. Furthermore, the mean value of the difference between $SnCl_2$ and its control (119.4±31.1/sec, N=8) was significantly different from that between $SnCl_4$ and its control (−4.5±7.4/sec, N=10) at $p<0.001$ by the simple *t*-test. There was no difference between the mean values of the controls for $SnCl_2$ and for $SnCl_4$ by the simple *t*-test.

Figure 2 shows the effects of $SnCl_2$ and $SnCl_4$ on the quantal content of the e. p. p.. Similarly to the effect on the m. e. p. p. frequency, $SnCl_2$ increased the quantal content whereas $SnCl_4$ did not significantly change it. Moreover, the mean value of the difference between $SnCl_2$ and its control (0.48 ± 0.14, N=10) was significantly different from that between $SnCl_4$ and its control (0.05 ± 0.07, N=15) at $p<0.001$ by the simple *t*-test. There was no difference between the mean values of the controls for $SnCl_2$ and for $SnCl_4$ by the simple *t*-test.

Discussion

Whereas $SnCl_2$ (30 μM) significantly increased both the m. e. p. p. frequency and the quantal content of the e. p. p., $SnCl_4$ (30 μM) had no effect on either of them. To examine whether the results obtained in the experiments examining the effects of $SnCl_2$ involved the actions of Sn^{4+}, we

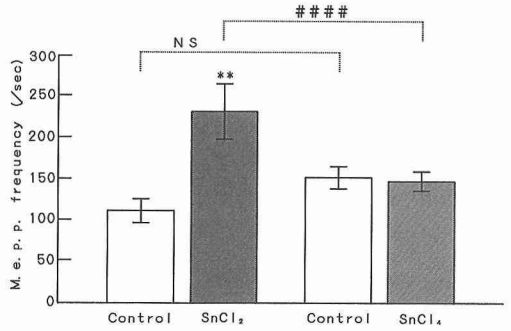

Fig. 1: Comparison of the effects of $SnCl_2$ and $SnCl_4$ on the m. e. p. p. frequency. Whereas $SnCl_2$ (30 μM) significantly increased the m. e. p. p. frequency, $SnCl_4$ (30 μM) did not change it. ** : $p<0.01$ compared to the control values. #### : $p<0.001$ compared to the difference of $SnCl_2$ from its control. There is no significant difference between the control values for $SnCl_2$ and for $SnCl_4$. NS : not significant.

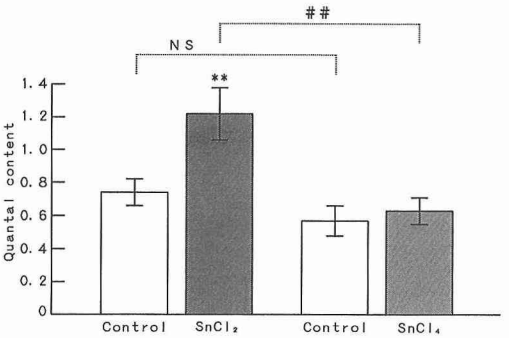

Fig. 2: Comparison of the effects of $SnCl_2$ and $SnCl_4$ on the quantal content of the e. p. p.. Whereas $SnCl_2$ (30 μM) significantly increased the quantal content, $SnCl_4$ (30 μM) did not change it. ** : $p<0.01$ compared to the control values. ## : $p<0.01$ compared to the difference of $SnCl_2$ from its control. There is no significant difference between the control values for $SnCl_2$ and for $SnCl_4$. NS : not significant.

statistically analyzed the mean values of the differences of $SnCl_2$ from its control and of $SnCl_4$ from its control. There were significant differences between the two both in the m. e. p. p. frequency and in the quantal content. The results obtained show that the actions of Sn^{4+} are not involved in the effects shown by $SnCl_2$. In conclusion, it is confirmed that $SnCl_2$ itself facilitates the transmitter release from the motor nerve terminals at the concentration of 30 μM.

Brûlé *et al.*[7] have reported that $SnCl_4$ (0.11 mM) has inhibitory effects on the electrical and mechanical activities of the crab skeletal muscle. It decreases the resting potential, height of the action potential, and twitch tension. In this study, however, we did not observe such inhibitory actions of $SnCl_4$. The low concentration of $SnCl_4$ used here (30 μM) may have precluded exertion of the properties. It is necessary to examine the effects of $SnCl_4$ of higher concentrations to determine whether $SnCl_4$ facilitates or inhibits the neuromuscular transmission.

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抄録：マウス運動神経末端における伝達物質遊離に対する塩化第一スズおよび塩化第二スズの作用

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齲蝕予防薬のフッ化第一スズは骨格筋の収縮を増強するが，塩化第一スズ($SnCl_2$)を用いた実験から，その作用にはフッ素イオンだけでなく第一スズイオン（Sn^{2+}）も関与しており，それは運動神経末端からの伝達物質遊離を促進することが推察された。ところでSn^{2+}は酸化され第二スズイオン（Sn^{4+}）となりやすい性質を有しており，これまで調べてきた$SnCl_2$の作用が全てSn^{2+}のものとは言いにくく，Sn^{4+}が関与している可能性も否定できない。そこで今回$SnCl_2$の伝達物質遊離促進作用を確かめるために$SnCl_2$と$SnCl_4$の作用とを比較した。

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。灌流液の組成(mM)はNaCl, 154；KCl, 5；$CaCl_2$, 2；$MgCl_2$, 1；glucose, 11およびHEPES, 5（pH＝7.3）であった。灌流液には実験中常に混合ガス（95%O_2＋5%CO_2）を通気した。3M 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。$SnCl_2$および$SnCl_4$は各々同濃度の酒石酸水溶液に溶解して適用した。

その結果$SnCl_2$（30 μM）はm. e. p. p. の発生頻度およびe. p. p. のquantal contentを有意に増大させたが，同濃度の$SnCl_4$では変化は見られなかった。更に$SnCl_2$の作用にSn^{4+}が関与していると仮定し，$SnCl_2$と$SnCl_4$の実験値と各々の対照値との差同志を比較したところ，m. e. p. p. 発生頻度およびquantal contentともに有意な差が見られた。以上のことからこれまでに得られた$SnCl_2$の結果にはSn^{4+}の作用は含まれておらず，$SnCl_2$が伝達物質遊離促進作用を有することが確かめられた。