[Original] Matsumoto Shigaku, 18 : 18~22, 1992

key words : stannous chloride — endplate potential — miniature endplate potential — rat

Effects of Stannous Chloride on the Rat Neuromuscular Transmission

TOSHIMI HATTORI and HIROSHI MAEHASHI

Department of Dental Pharmacology, Matsumoto Dental College

(Chief : Prof. H. Maehashi)

Summary

Stannous chloride ($SnCl_2$) facilitates frog neuromuscular transmission. Effects of $SnCl_2$ on the neuromuscular junction of the rat have been investigated to determine whether mammalian responses to $SnCl_2$ are the same as those of amphibians. $SnCl_2$ (30 μM) had no effect on the resting potential or the membrane resistance of the muscle fiber. $SnCl_2$ did not change the amplitude of the endplate potential. $SnCl_2$ significantly raised the frequency of the miniature endplate potential in the high potassium-medium. $SnCl_2$ decreased the amplitude of the miniature endplate potential. These results suggest that $SnCl_2$ may facilitate the transmitter release from the nerve terminals, but it may reduce the acetylcholine sensitivity of the endplate in the rat.

Introduction

In the frog neuromuscular junction, stannous chloride ($SnCl_2$) enhances the calcium (Ca) entry into the nerve terminals by activating the N-type Ca channels. This augmenting effect of $SnCl_2$ is depressed by ω-conotoxin GVIA (ω-CT), a blocker of L- and N-type Ca channels[1]. It is known that there is difference in the sensitivity of the Ca channel to drugs between the animal species[2~4]. For example, Olivera *et al.*[2] have reported that ω-CT blocks presynaptic Ca channels of the frog neuromuscular junction but intraperitoneal administration of ω-CT has no effect on the mouse. Suszkiw *et al.*[3] have described that ω-CT at the concentration higher than 0.3 μM completely inhibits Ca entry in the frog and chick brain synaptosomes but was only partly effective in blocking Ca influx in the rat preparations. Thus, the present study has been undertaken to determine whether or not there is difference between the actions of $SnCl_2$ on the frog (amphibian) and those on the rat (mammalian).

Materials and Methods

Phrenic nerve-diaphragm preparations from Wistar male rats (body weight, 200—250 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 (N—(2—Hydroxyethyl) piperazine-N'-2-ethanesulfonic acid), 5[5]. pH was adjusted at 7.3. By conventional

(accepted for publication on January 8, 1992)

intracellular recording method with glass microelectrodes, endplate potentials (e. p. p.) and miniature endplate potentials (m. e. p. p.) at the neuromuscular junction and resting potentials (r. p.) and membrane resistances (m. r.) of the muscle fiber were measured[6]. The m. r. was obtained from dividing the peak height of electrotonic potential by the strength of the current passed through the cytoplasmic membrane[7]. To record the e. p. p., *d*-tubocurarine at the concentration of 1.0 μM was added to the perfusate. Isotonicity of high potassium (K)-medium containing KCl 5 times as much as the normal saline was maintained by reduction in NaCl concentration. Concentration of $SnCl_2$ applied in the present study was 30 μM which significantly facilitates the frog neuromuscular transmission[6].

Chemicals and drugs used in this study were $SnCl_2$ and *d*-tubocurarine chloride obtained from Nacalai Tesque (Japan). Statistical analyses of the data were peformed by the Student's 2-sided paired *t*-test. Differences between mean values obtained before and after $SnCl_2$ application were considered significant if the probability of error was less than 0.05.

Results

$SnCl_2$ (30 μM) had no effect on the r. p. or the m. r.. The values obtained immediately before and 3 min after $SnCl_2$ application were as follows, respectively : r. p. (mV), 83.39±3.11 and 81.44±3.19 (N=6) ; m. r. ($\times 10^5\Omega$), 7.69±0.60 and 7.07±0.83 (N=9). Each value represents the mean value±the standard error and N the number of experiments. These r. p. and m. r. were calculated by using the mean values of 11 to 17 data obtained from one experiment.

As shown in Fig. 1, $SnCl_2$ (30 μM) did not change the e. p. p. amplitude at all. The values (mV) obtained before and after $SnCl_2$ application were 3.76±0.33 and 3.56±0.30 (N=18).

Effects of $SnCl_2$ on the m. e. p. p. were examined both in the normal saline and in the high K-one. Under the former condition, $SnCl_2$ (30 μM) decreased the m. e. p. p. frequency (/sec) from 1.77±0.26 to 1.40±0.23 (N=7), as shown in Fig. 2.

Under the latter condition, on the contrary, $SnCl_2$ (30 μM) significantly raised it from 26.09± 5.36 to 41.53±6.00/sec (N=12), as shown in Fig. 3A. Fig. 3B illustrates the effect of $SnCl_2$ on the m. e. p. p. amplitude in the high-K medium. $SnCl_2$ (30μM) decreased it from 0.29±0.02 to 0.26±0.01 mV (N=7), suggesting that $SnCl_2$ may reduce the acetylcholine (ACh) sensitivity of the endplate[8].

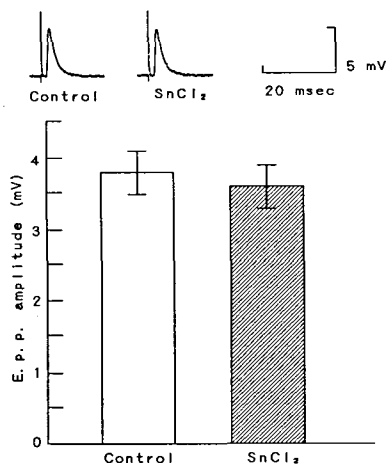

Fig. 1 : Non-effect of $SnCl_2$ on the rat e. p. p. Upper figures : Wave forms recorded immediately before and 3 min after $SnCl_2$ (30μM) application. Lower figures : Comparison between the e. p. p. amplitudes before and after the application. Columns and vertical bars represent the mean value and the standard error.

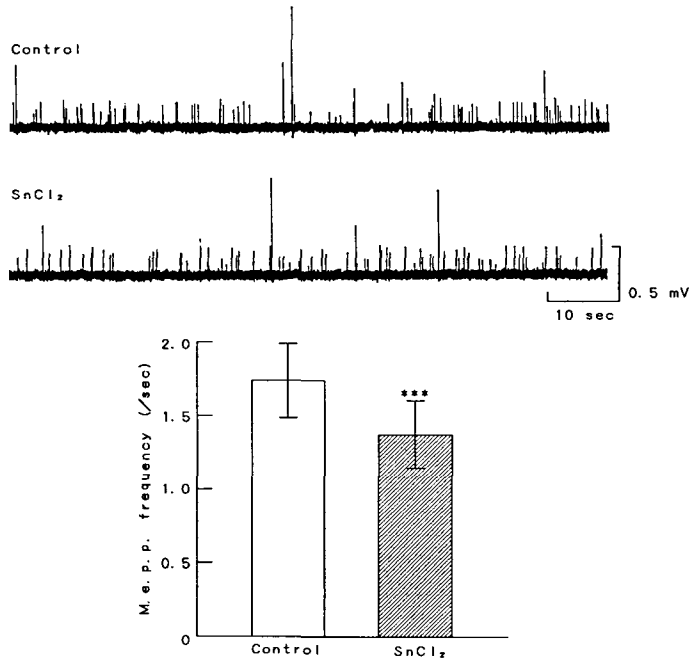

Fig. 2: Decrease in the m. e. p. p. frequency by $SnCl_2$ in the normal saline. $SnCl_2$ (30μM) decreased the m. e. p. p. frequency. * * * : Significantly different from the value obtained before the application at $p<0.005$.

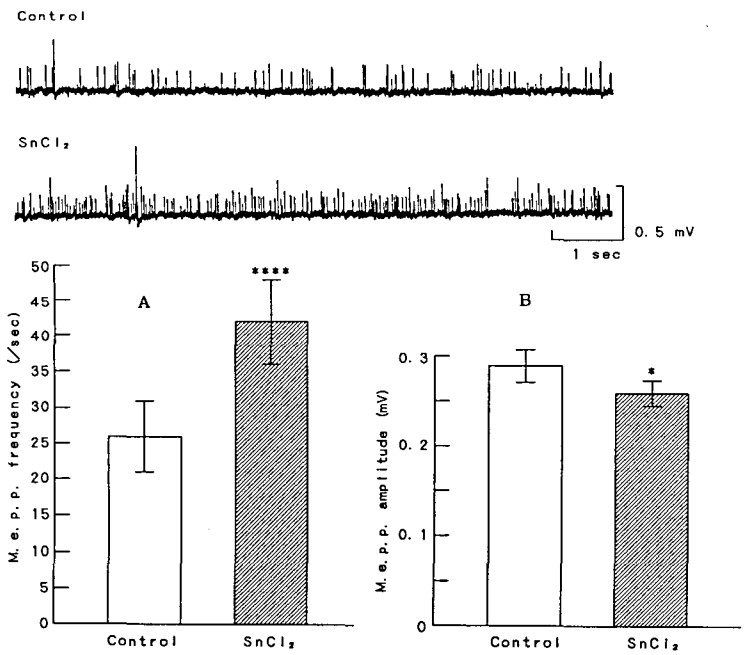

Fig. 3: $SnCl_2$-induced rise in the m. e. p. p. frequency and reduction in the m. e. p. p. amplitude. $SnCl_2$ (30μM) significantly raised the m. e. p. p. frequency (A) but reduced the m. e. p. p. amplitude (B). The high K-saline was used as the perfusate. * and * * * *: Significantly different from the value obtained before the application at $p<0.05$ and $p<0.001$, respectively.

Discussion

Sano *et al.*[4] have observed that ω-CT decreases the peak height of the e. p. p. in the frog but has no effect on the mouse neuromuscular junction. Consequently, they have described that this high specificity of the effect of ω-CT may be due to the structure of Ca channels in various species. Thus, from the fact that $SnCl_2$ antagonizes ω-CT[1], we speculate that $SnCl_2$ has the different effects on the rat from those on the frog, too. The present study has been conducted to compare the responses of the rat to $SnCl_2$ with those of the frog.

The $SnCl_2$ raised the m. e. p. p. frequency in the high K-medium in the rat similarly to in the frog. This result shows that $SnCl_2$ facilitates the transmitter release from the nerve terminals[8]. $SnCl_2$ had no augmenting effect on the e. p. p.. This is extremely different from the response of the frog[6]. However, these responses are not incompatible with each other. $SnCl_2$ decreased the m. e. p. p. amplitude, indicating that $SnCl_2$ decreased the sensitivity of ACh receptor on the endplate[8]. Thus, these results can be reasonably explained as follows: As the result that the transmitter release from the presynaptic membrane was enhanced but the ACh sensitivity of the postsynaptic membrane was lowered, the e. p. p. amplitude seems likely to have maintained constant. Although the r. p. and the m. r. of the muscle fiber influence the e. p. p. and the m. e. p. p. amplitudes[7], the r. p. and the m. r. remained unchanged. Therefore, the r. p and the m. r. are not related to the constant-maintained e. p. p. and to the decrease in the m. e. p. p. amplitude. Although $SnCl_2$ unaffected the frog m. e. p. p. frequency in the normal saline[1], in the rat, $SnCl_2$ decreased it under the same condition contrary to our expectation. This difference suggests the possibility that $SnCl_2$ might exert its new properties in the rat in the normal saline or that tartaric acid, a solvent for $SnCl_2$[6], might be involved in this reduction. We have an intention to find out the factors reducing the m. e. p. p. frequency under the normal condition.

The findings mentioned above show that $SnCl_2$ has an excitatory effect on the presynaptic membrane but, on the other hand, it has an inhibitory effect on the postsynaptic membrane, that is, $SnCl_2$ facilitates the transmitter release from the nerve terminals but reduces the sensitivity of the ACh receptor on the endplate. Further investigations are needed to determine whether the difference between the responses of the frog to $SnCl_2$ and those of the rat is due to animal-specific heterogeneity.

References

1) Hattori, T. and Maehashi, H. (1991) Activation of N-type calcium channels by stannous chloride at frog motor nerve terminals. Res. Commun. Chem. Pathol. Pharmacol. **145**: 125—128.
2) Olivera, B. M., Gray, W. R., Zeikus, R., McIntosh, J. M., Varga, J., Rivier, J., de Santos, V. and Cruz, L. J. (1985) Peptide neurotoxins from fish-hunting cone snails. Science, **230**: 1338—1343.
3) Suszkiw, J. B., Murawsky, M. M. and Fortner, R. C. (1987) Heterogeneity of presynaptic calcium channels revealed by species differences in the sensitivity of synaptosomal ^{45}Ca entry to ω-conotoxin. Biochem. Biophys. Res. Commun. **145**: 1283—1286.
4) Sano, K., Enomoto, K. and Maeno, T. (1987) Effects of synthetic ω-conotoxin, a new type Ca^{2+} antagonist, on frog and mouse neuromuscular transmission. Eur. J. Pharmacol. **141**: 235—241.
5) Molgó, L. and Mallart, A. (1988) The mode of action of guanidine on mouse motor nerve terminals. Neurosci. Lett. **89**: 161—164.
6) Hattori, T. and Maehashi, H. (1988) Anti-curare action of stannous ion in the frog neuromuscular junction. Life Sci. **42**: 2639—2644.
7) Katz, B. and Thesleff, S. (1957) On the factors which determine the amplitude of the 'miniature

end-plate potential'. J. Physiol. **137**: 267—278.
8) Katz, B. (1962) The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. *Proc. Roy. Soc. Biol.* **155**: 455—477.

抄録：ラット神経筋伝達に対する塩化第一スズの作用

服部敏己，前橋　浩（松本歯大・歯科薬理）

フッ化第一スズの骨格筋興奮作用における第一スズイオンの関与様式を明らかにするために塩化第一スズ($SnCl_2$)の作用について調べた結果，カエルでは運動神経末端においてN型のカルシウム(Ca)チャネルを介したCa流入を増大させることにより神経筋伝達を促進することがわかっている．ところでCaチャネルの薬物感受性には動物種差のあることが報告されている．今回は材料をラット（温血動物）に換えることにより$SnCl_2$に対するカエル(冷血動物)の反応との違いの有無を調べることを目的として実験を行なった．

材料には雄性Wistar系ラット（体重：200～250 g）の横隔膜神経筋標本を用いた．3 M KClを充塡したガラス微小電極により終板電位（e. p. p.），微小終板電位（m. e. p. p.），筋線維の静止膜電位（r. p.）および膜抵抗（m. r.）を測定した．e. p. p.記録に際しては *d*-tubocurarine 1.0 μM を灌流液に添加した．m. e. p. p.の記録は正常灌流液および高カリウム（K）-灌流液（正常濃度の5倍のKClを含む）を使った場合について調べた．$SnCl_2$濃度はカエルの神経筋伝達を有意に促進する30 μM を用いた．

$SnCl_2$はr. p.およびm. r.に対してカエルの場合と同様に変化させなかった．カエルにおいては顕著な増大が見られたe. p. p.振幅を$SnCl_2$は全く変化させなかった．m. e. p. p.発生頻度に対して$SnCl_2$は正常灌流液では低下させ（対照値の0.80倍），高K-灌流液ではカエルと同様に著明に上昇させた（1.59倍）．$SnCl_2$はm. e. p. p.振幅を減少させた（0.90倍）．以上の結果より，$SnCl_2$はカエルの神経筋接合部では神経末端だけに作用したのに対して，ラットでは神経末端において伝達物質の遊離量を増加させるが，一方，筋終板にも作用してacetylcholine receptorの感受性を低下させていることが考えられる．