INHIBITION OF K-INDUCED TONIC CONTRACTION IN VAS DEFERENS OF GUINEA PIG BY CHLORPROMAZINE AND ITS FREE RADICAL

Shigeru YAMABE, Aritomo SUZUKI and Hiroshi MATSUMOTO

Research Institute, Kobe College, Nishinomiya and Department of Pharmacology, Faculty of Medicine, Kobe University, Kobe, Japan

Accepted July 3, 1973

Abstract—Inhibitory effects of chlorpromazine (CP) and its free radical (CP•) on the tonic component of the K-contraction of vas deferens isolated from guinea pig were studied by the Magnus method. CP and CP• were equally effective at concentrations higher than $2 \times 10^{-7}$ g/ml for selective inhibition of the tonic component in neutral media (pH 7.4). The effect of CP• was 100 times greater than that of CP in acidic media (pH 5.0). These findings can be explained in terms of the increased stability of CP•, since the absorption spectral analysis showed that CP• was apparently stable in acidic media. Among reducing agents studied, 2-mercaptoethylamine was the most potent in restorative activity for the above inhibition.

In a previous paper (1) the authors reported that the K-contraction (induced by high concentration of KCl) in isolated vas deferens from guinea pig has a phasic and a tonic component. The former was due to Ca-release from Ca-stores in the membrane, while the latter was due to major Ca-influx by an active transport mechanism with minor Ca-release. Similar experimental results and their interpretation in terms of Ca-supply as related to the mechanism of K-contraction with other smooth muscles have been reported by several workers (2-4).

Chlorpromazine (CP) is a useful agent not only as a major tranquilizer but also as an enzyme inhibitor of (Na + K)-ATPase (5), glutamate dehydrogenase (6) and the other enzymes (7, 8). According to studies by Akera and Brody, (Na + K)-ATPase in rat brain homogenate was more strongly inhibited by CP free radical (CP•) than CP in vitro experiments. Thus, it is of interest to study the effects of CP and CP• on the tonic component of the K-contraction, since this component may require the free energy liberated from the ATP-ATPase system for active transport of Ca to maintain the contraction. This paper describes: (i) inhibition of the tonic component of the K-contraction by CP and CP•, (ii) selective increase in the inhibitory effect of CP• in acidic media, and (iii) restorative action of several reducing agents on the inhibited tonic contraction.

MATERIALS AND METHODS

Vas deferens was isolated from male guinea pig and suspended in 50 ml organ bath containing Locke's solution. The suspension medium was continuously aerated and maintained at a temp. of 30°C. The pH was adjusted to 7.4 in the neutral medium ex-
periments and 5.0 in the acidic ones. Changes in the muscle tension were recorded isotonically by the Magnus method. K-contraction was induced by the addition of \(5 \times 10^{-4}\) g/ml KCl.

Chlorpromazine hydrochloride (CP) was purchased from Wako Pure Chemicals and 2-mercaptopropionylglycine (trade name: Thiola) was kindly provided by Santen Pharmaceutical Company.

The CP· was prepared by the addition of \(2 \times 10^{-4}\) g/ml Ce(SO₄)₃ to the suspension medium, containing a given concentration of CP. The formation of CP· was confirmed by the characteristic absorption peaks (277 and 526 nm) in the ultraviolet and visible regions. Using the time course of absorbance at 526 nm, the stability of CP· was compared at pH 7.4 and 5.0.

**RESULTS**

**Effect of pre-treatment with CP in a neutral medium**

Typical data of the effect of pre-treatment with CP on the K-contraction in a neutral medium (pH 7.4) are shown in Fig. 1. Curve a is a standard K-contraction. By the addition of a high concentration of KCl to the suspension medium, a strong phasic contraction was induced and was followed by a strong tonic contraction. The latter was maintained for more than one hr in a normal suspension medium. Such a K-contraction was markedly affected by pre-treatment with CP (10⁻⁵ g/ml) in a neutral medium, as shown in curve b. Time course of the change in the K-contraction following the CP-pre-treatment is shown in curves c, d and e. In this time course, the tonic contraction was preferentially inhibited and restoration was not observed even after a long lapse of time. On the other hand, the phasic contraction was gradually restored though it was strongly inhibited by the CP-pre-treatment in an early period.

![Fig. 1. Effect of pre-treatment with CP on the K-contraction in a neutral medium.](image)

(a) : Control curve induced by KCl (5 x 10⁻² g/ml),
(b)~(e) : tension change curves affected by pre-treatment with CP (10⁻⁵ g/ml)
(at 10 min, 1 hr, 2 hr and 3 hr after pre-treatment).

**Effects of CP and CP· in a neutral medium**

Inhibitory effects of CP and CP· on the tonic component in a neutral medium (pH 7.4) are shown in Fig. 2. It is apparent from curves a and b that there was no difference in the inhibitory effect between CP and CP·, the minimum effective concentration being \(2 \times 10^{-4}\) g/ml. These inhibitory effects were equally enhanced with increase of the concentration of CP and CP· as shown in curves c and d, respectively, where the concentrations were \(10^{-5}\) g/ml.
CONTRACTION INHIBITION BY CHLORPROMAZINE

**Fig. 2.** Effects of CP and CP• on the K-contraction in a neutral medium.
(a) : CP (2 × 10⁻⁶ g/ml),
(b) : CP• (2 × 10⁻⁴ g/ml Ce(SO₄)₂ added),
(c) : CP (10⁻⁶ g/ml),
(d) : CP• (2 × 10⁻⁴ g/ml Ce(SO₄)₂ added).

**Fig. 3.** Effects of CP and CP• on the K-contraction in an acidic medium.
(a) : CP (2 × 10⁻⁶ g/ml) and then CP• (2 × 10⁻⁴ g/ml Ce(SO₄)₂ added)
(b) : CP• and then CP (concentrations were the same).

### Effects of CP and CP• in an acidic medium

The inhibitory effects of CP and CP• in an acidic medium (pH 5.0) are shown in Fig. 3. Contrary to the effects in the neutral medium, there was a marked difference in inhibitory activity between CP and CP•, the latter being 100 times stronger than the former. Typical data among several tracings are presented in curves a and b, where the concentrations of CP and CP• (as concentration of CP) were 2 × 10⁻⁷ g/ml. In curve b, CP• was added before the CP-addition, while the order of addition was inversed in curve a.

### Effect of Ce(SO₄)₂ in a neutral and an acidic medium

Fig. 4 shows effects of Ce(SO₄)₂ on the K-contraction. It is clear from two representative tracings that Ce(SO₄)₂ was ineffective at concentrations below 2 × 10⁻⁵ g/ml. This concentration is much higher than that employed in the experiments for Figs. 2 and 3.
Effects of reducing agents in a neutral medium

Figs. 5 and 6 show restorative effects of two reducing agents, cysteine and 2-mercapto-propionylglycine. In both cases the time schedule and conditions in experiments as CP-pre-treatment (30 min-incubation of $10^{-5}$ g/ml CP), washing, and restoration by a reducing agent, were identical and are indicated on the tracing as arrows. The addition of a
reducing agent (10⁻³ g/ml) and subsequent 30 min-incubation was performed after the phasic component was completely recovered. It is apparent from the figures, that the extinction of the tonic component of the K-contraction by CP-pre-treatment could be restored to the level of approx. 50% inhibition.

Absorption spectral analysis of CP• formation

The absorption spectra of CP• prepared by Ce(SO₄)₂ oxidation show two groups of absorption peaks, one at 270, 277, and 526 nm, and the other at 296 and 340 nm, the former being characteristic of CP• and the latter of CP sulfoxide, as reported by Akera and Brody (5). It is apparent that the height of the main peak at 526 nm decreased over a lapse of time, and rate was accelerated with increase of pH value, indicating a progressive decrease in the stability of CP•. The half-life of CP• was about 6 min at pH 5.0, while it was quite short-lived at pH 7.4.

DISCUSSION

It has been well established that Ca plays an important role in excitation-contraction coupling in muscle contractions. Irakawa and Holland (9) reported that an enhanced Ca uptake was observed in both the phasic and the tonic contractions induced by KCl in taenia coli and a significant rise of tissue Ca was simultaneously observed only in the tonic contraction. These findings indicate that in the phasic contraction, sufficient Ca is released from a cellular site (Ca-store) to initiate contraction, whereas in the tonic contraction, sufficient Ca has to cross the membrane for contraction. Thus this transmembrane-transport of Ca may be correlated to the metabolism in the cellular membrane.

Pfaffman, Urakawa, and Holland (10), using various inhibitors of metabolism and active transport, showed experimental evidence concerning metabolism dependency of the above transport. From their observation that inhibition of metabolism by Li and ouabain abolished the tonic contraction, while having no effect on the phasic contraction, they concluded that the phasic response is a passive process resulting from a release of tissue Ca, while the tonic one is an active process resulting from a K-induced (Na—Ca)—linked transmembrane transport. Based on the principles of bioenergetics, such an active transport would require the energy liberated from ATP by (Na—Ca)—ATPase.

Among the findings concerning enzyme inhibition by CP, an important one is that CP inhibited (Na+K)—ATPase from rat brain homogenate, and this inhibition was enhanced when CP was irradiated by ultraviolet light. This finding shows that the inhibitory activity of CP• is much stronger than that of CP, since absorption spectral analysis proves a temporary formation of CP• even in such a neutral medium as pH 7.4. As CP inhibits several different enzyme systems, the inhibition of (Na—Ca)—ATPase by CP appears feasible. Our present findings in the neutral media, where CP inhibited selectively the tonic component of K-induced contraction support this supposition.

Furthermore, our findings that, in acidic media, the in vivo activity of CP• was markedly enhanced, might be well explained in terms of increased stability of CP•, and, therefore, may support those of Akera and Brody concerning the stronger inhibition of CP•.
in vitro experiments. Thus, it seems likely that CP affects its inhibition of ATPase after it is converted into CP• by a biological oxidation mechanism in the membrane.

As reported by Akera and Brody (11), the inhibition of (Na + K)-ATPase by CP• was apparently restored by various reducing agents, cysteine being the most potent and L-ascorbic acid being ineffective. The reason for such a difference between the reducing agents is obscure, yet our experimental data show also a restorative activity of cysteine and a more potent one of 2-mercaptoethanol. These findings indicate an oxidative inactivation of ATPase (presumably, its active SH groups) for the mechanism of inhibition of the tonic contraction by CP and CP•, but further studies are required before the intrinsic mode of action in vivo as well as in vitro can be elucidated.

REFERENCES
1) Suzuki, A. and Matsumoto, H.: Folia Pharmacol. japon. 67, 218P (1971) (in Japanese)
2) Chujo, N. and Holland, W.C.: Am. J. Physiol. 205, 94 (1963)
3) Shanes, A.M.: J. cell. comp. Physiol. 57, 193 (1961)
4) Karaki, H., Ikeda, M. and Uraikawa, N.: Japan. J. Pharmacol. 17, 603 (1967)
5) Akera, T. and Brody, T.M.: Mol. Pharmacol. 4, 600 (1968)
6) Fain, L.A. and Shemisa, O.: Mol. Pharmacol. 6, 156 (1970)
7) Abood, L.G.: Proc. Soc. exp. Biol. Med. 88, 688 (1955)
8) Löw, H.: Biochim. biophys. Acta 32, 11 (1959)
9) Uraikawa, N. and Holland, W.C.: Am. J. Physiol. 207, 873 (1964)
10) Peffman, M., Uraikawa, N. and Holland, W.C.: Am. J. Physiol. 208, 1203 (1965)
11) Akera, T. and Brody, T.M.: Mol. Pharmacol. 5, 685 (1969)