The Effect of Trifluoperazine on Muscle Tension and Cytoplasmic Ca^{2+} Level in Guinea Pig Ileum

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Abstract—In guinea pig ileal muscle strips, trifluoperazine (TFP) inhibited the high K^+ -induced contraction with an IC_{50} of $1.9 \mu\text{M}$ and the Ca^{2+} -induced contraction in skinned fiber with an IC_{50} of $44 \mu\text{M}$. TFP decreased the high K^+ -induced increase in cytoplasmic Ca^{2+} level at the concentration $3 \mu\text{M}$, which did not inhibit the contraction in skinned fiber. It is suggested that the inhibitory effect of TFP on the high K^+ -induced contraction is mainly due to the decrease in cytoplasmic Ca^{2+} .

Trifluoperazine (TFP) has been shown to be a calmodulin inhibitor (1, 2). In electrophysiological studies, it has been shown that TFP inhibits the spontaneous electrical activity (3) and the inward Ca^{2+} or Ba^{2+} currents (4, 5) in heart cells and smooth muscle cells, suggesting that TFP inhibits Ca^{2+} influx through Ca^{2+} channels. In order to evaluate this possibility, we compared the inhibitory effect of TFP in the intact tissue and skinned fibers prepared from the ileal longitudinal smooth muscle of guinea pig. Furthermore, we compared the effect of TFP on the cytoplasmic free Ca^{2+} level ($[\text{Ca}^{2+}]_{\text{cyt}}$) in smooth muscle with those of a Ca^{2+} channel blocker, nifedipine, and an aerobic metabolic inhibitor, KCN.

Male guinea pigs weighing 300–400 g were stunned and bled. A portion of the ileum beginning 10 cm above the ileo-caecal junction was removed, and the longitudinal smooth muscle layer was separated from the underlying circular layer. Muscle strips of about 5 mm width and 15 mm length were used for measurement of tension and $[\text{Ca}^{2+}]_{\text{cyt}}$. Ileal strips of 0.5 mm width and 10 mm length were skinned with 1% Triton X-100 as described by Rüegg et al. (6). $[\text{Ca}^{2+}]_{\text{cyt}}$ in the smooth muscle strip was recorded simultaneously with muscle tension, using the fluorescent indicator fura-2 (7, 8). Briefly, the

muscle strip was treated with 1 ml of physiological salt solution (PSS) containing $5 \mu\text{M}$ acetoxymethyl ester of fura-2 (fura-2/AM) and 0.25% cremophor EL, a non-cytotoxic detergent, for 6 hours at room temperature. After rinsing the fura-2-loaded muscle with PSS, one end of the muscle strip was fixed with minute stainless steel-pins on the bottom of an organ bath (7 ml) at 37°C , and the other end was connected to an isometric force transducer (Nihon Kohden). The excitation light was obtained from a xenon high pressure lamp (75W) equipped with a rotating filter wheel (48 Hz) containing 340 nm or 380 nm (± 5.5 nm) interference filters. The emitted light from the muscle strip was collected by a photomultiplier through a 500 nm (± 10 nm) bandpass filter and measured at 0.25 sec time constant by a fluorimeter (CAF 100, Japan Spectroscopic). The ratio of the fluorescence due to excitation at 340 nm and 380 nm was calculated from successive illumination periods and the isometric muscle tension was simultaneously recorded.

PSS used to measure muscle tension contained: 136.9 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl_2 , 1.0 mM MgCl_2 , 11.9 mM NaHCO_3 and 5.5 mM glucose; and that used to measure $[\text{Ca}^{2+}]_{\text{cyt}}$ contained: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl_2 , 1.0 mM MgCl_2 , 11.9 mM NaHCO_3 , 5.5 mM glucose,

0.01 mM EDTA and 0.01 mM N,N,N',N'-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN), a chelator of heavy metal ions. These solutions were aerated with 95% O₂ and 5% CO₂ at 37°C and pH 7.2. High K⁺ solution was made by adding 60 mM KCl (Hyper-60K⁺) which was used in the measurement of muscle tension or by replacing 68.5 mM NaCl with equimolar KCl (Iso-70K⁺) which was used in the measurement of [Ca²⁺]_{cyt} because hyperosmolarity interferes with the fura-2 fluorescence (8). Skinned fibers were bathed at 24°C in a relaxing solution containing 50 mM KCl, 20 mM imidazole, 2 mM EGTA, 8 mM MgCl₂, 5 mM ATP, 3 mM KH₂PO₄, 3 mM K₂HPO₄, 0.1 μM calmodulin and 1 mM NaN₃, adjusted to pH 6.7 with KOH. The drugs used were fura-2/AM (Dojin), cremophor EL (Nakarai Chemicals), TPEN (Dojin), nifedipine (Sigma) and TFP (Sigma).

Measurements of muscle contraction indicated that TFP (0.1 μM–100 μM) inhibited the contractions induced by Hyper-60K⁺ (Fig. 1a), with an IC₅₀ (concentration needed to induce 50% inhibition) of 1.9 μM. TFP (1 μM–100 μM) also relaxed the 1 μM Ca²⁺-induced tonic contraction in chemically skinned fiber, with an IC₅₀ of 44 μM (Fig. 1a).

Simultaneous recordings of muscle tension and [Ca²⁺]_{cyt} in ileal strips showed that nifedipine (0.1 μM) completely inhibited

the Iso-70K⁺-induced tonic contraction, whereas the [Ca²⁺]_{cyt} was inhibited by about 70% (Fig. 2a). In contrast to this, 1 mM KCN strongly inhibited the Iso-70K⁺-induced contraction without inhibiting the [Ca²⁺]_{cyt} (Fig. 2b). TFP (3 μM) induced a gradual decrease in both tension and [Ca²⁺]_{cyt} stimulated by Iso-70K⁺ (Fig. 2c). The inhibitory effect of TFP on [Ca²⁺]_{cyt} was concentration-dependent (Fig. 1b). Comparing the inhibitory effects of TFP on muscle tension (Fig. 1a) and [Ca²⁺]_{cyt} (Fig. 1b), TFP seemed to inhibit muscle contraction more strongly than [Ca²⁺]_{cyt}, as was the case with nifedipine (Fig. 2a). TFP is a fluorescent substance which increases its fluorescence due to excitation at 340 nm at high concentrations (10 μM–50 μM) but not at 3 μM. An application of EGTA (2 mM) decreased the [Ca²⁺]_{cyt} to or below the resting level (Fig. 2a–c).

In the present experiments, it was found that although KCN strongly inhibited the high K⁺-induced contraction, it did not change [Ca²⁺]_{cyt}. This results suggested that the inhibition of aerobic metabolism uncouples muscle contraction from the increase in [Ca²⁺]_{cyt}. In contrast to this, the relaxant effect of TFP accompanied the decrease in [Ca²⁺]_{cyt}, and this effect was quite similar to that of nifedipine. It was also found that the IC₅₀ of TFP in the intact tissue was approximately 1/10 of that in the skinned

Fig. 1. The inhibitory effects of TFP on contractions in intact or skinned tissue (a) and [Ca²⁺]_{cyt} in fura-2-loaded tissue (b). (a) Ordinate: Relative tension (%) of the contraction due to Hyper-60K⁺ solution or 1 μM Ca²⁺. Abscissa: Logarithmic concentrations (-log M) of TFP. (b) Ordinate: The percent of fluorescent ratio due to an application of 2 mM EGTA and Iso-70K⁺ solution were taken as 0% and 100%, respectively. Abscissa: Each point is obtained from different muscle strip.

Fig. 2. Typical recording of fluorescence due to excitation at 340 nm (F340) or 380 nm (F380), the fluorescent ratio (Ratio) and muscle tension (Tension) simultaneously measured in the ileal longitudinal muscle strips loaded with fura-2. The effect of 0.1 μ M nifedipine (a), 1 mM KCN (b) and 3 μ M TFP (c) on the Iso-70K⁺-induced contraction. The vertical bars present the percent change in fluorescence, taking the fluorescence without excitation lights as 0% and that with 340 nm or 380 nm excitation light in resting muscle as 100%.

fibers. These results suggest that the inhibitory effect of TFP is not attributable to the inhibition of contractile elements but to the decrease in $[Ca^{2+}]_{\text{cyt}}$ in intact tissues. These results are consistent with the finding that TFP inhibits Ca^{2+} channels in cardiac and smooth muscle cells (3–5).

Both TFP and nifedipine inhibited muscle contraction more strongly than $[Ca^{2+}]_{\text{cyt}}$. This result may indicate the decrease in Ca^{2+} -sensitivity of contractile elements induced by these inhibitors. Further studies are necessary to clarify this phenomenon.

In conclusion, it is suggested that TFP as well as nifedipine inhibits the high K⁺-induced contraction predominantly by the decrease in $[Ca^{2+}]_{\text{cyt}}$, whereas KCN inhibits contraction by the inhibition of the contractile elements in the guinea pig ileal longitudinal smooth muscle.

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