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Contractile Action of Mn^{2+} via Ca^{2+} Channels Activated by Bay K 8644 in Guinea-pig Taenia Coli

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Abstract

Mn^{2+} has been shown to inhibit K^+-induced contraction of smooth-muscle, to induce contraction of smooth-muscle in Ca^{2+}-free, K^+ medium and to activate the contractile proteins of skinned fibres of smooth muscle cells. Further work has suggested that Mn^{2+} penetrates the cytoplasm through voltage-dependent Ca^{2+} channels when the cell membranes of smooth muscles are depolarized with K^+. We have investigated whether in Ca^{2+}-free medium, Mn^{2+} enters the cytoplasm through Ca^{2+} channels and induces contraction of guinea-pig taenia coli in the presence of Bay K 8644, a dihydropyridine Ca^{2+}-channel agonist which prolongs the open state of the voltage-dependent Ca^{2+} channels in smooth-muscle cells.

In Ca^{2+}-free medium the application of 5 mM Mn^{2+} in the presence of Bay K 8644 caused contraction of and concomitant increase in Mn^{2+} uptake in guinea-pig taenia coli smooth muscle. In the presence of Bay K 8644 nifedipine, a dihydropyridine Ca^{2+} channel antagonist, dose-dependently inhibited both manganese uptake and the contraction induced by Mn^{2+}.

These results suggest that Mn^{2+} enters the cytoplasm through dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels activated by Bay K 8644 and then induces contraction in taenia coli.

We have previously reported that in guinea-pig ileal longitudinal muscle, 5 mM manganese ion (Mn^{2+}) inhibited completely K^+ (60 mM)-induced contraction in normal Ca^{2+} medium and that the tension progressively increased to above the level of the original response to the K^+ after 3 h (Nasu et al 1994). Ushijima & Gomi (1991) reported that in the guinea-pig vas deferens the inhibitory effect of Mn^{2+} on K^+ (100 mM)-induced contractions was progressively reduced by repeated applications of K^+ in the presence of Mn^{2+}.

In Ca^{2+}-free, K^+ medium Mn^{2+} has also been shown to induce contraction of isolated smooth muscles such as the ileum (Nasu et al 1995; Nasu & Shibata 1997), aorta (Shibata 1969) and uterus (Sakai & Uchida 1981). The increase in manganese uptake induced by Mn^{2+} in a Ca^{2+}-free, K^+ medium was inhibited by nifedipine, a dihydropyridine Ca^{2+}-channel antagonist, in the guinea-pig ileum (Nasu et al 1995) and by diltiazem in the guinea-pig vas deferens (Tsunobuchi & Gomi 1990). These results suggest that Mn^{2+} penetrates the cytoplasm through voltage-dependent Ca^{2+} channels when the cell membranes of smooth muscles are depolarized with K^+. Incidentally, it has been shown that Mn^{2+} directly activates the contractile proteins of skinned fibres of smooth muscle cells (Savineau et al 1988).

Bay K 8644, a structural analogue of nifedipine, has been shown by the patch-clamp technique to prolong the open state of the voltage-dependent Ca^{2+} channels in smooth-muscle cells including colon (Xiong et al 1995), artery (Nelson & Worley 1989; Matsuda et al 1990) and trachea (Green et al 1993), and that this can be competitively inhibited by nifedipine.

In this work we have investigated whether in Ca^{2+}-free medium, Mn^{2+} enters the cytoplasm through the Ca^{2+} channels and induces contraction of guinea-pig taenia coli in the presence of Bay K 8644. The actions of Bay K 8644 on the responses by Mn^{2+} were compared with those obtained with K^+. We also used nifedipine to gain further infor-

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mation about the response by Mn^{2+} in the presence of Bay K 8644.

Materials and Methods

Chemicals

Bay K 8644 was obtained from Funakoshi (Tokyo, Japan) and nifedipine from Wako (Osaka, Japan). Other chemicals were of analytical grade. Bay K 8644 was first dissolved in 99·5% ethanol and then further diluted with distilled water; the final concentration of ethanol did not exceed 0·01%. All experiments were conducted in the dark to avoid light-induced degradation of the drugs.

Preparation, physiological solution and tension recording

Strips of taenia coli were isolated from the caecum of male Hartley-strain guinea-pigs, 400 g, and were immersed in modified normal Tyrode's solution saturated with 100% O_2 at 37°C. The solution contained (mM): NaCl 123·7, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, tris(hydroxymethyl)aminomethane 25 and glucose 5·5. The pH of the solution was adjusted to 7·4 with HCl at 37°C.

The muscle strips were suspended at a resting tension of 0·6 g and left to equilibrate for 40 min with several changes of the Tyrode's solution. Isometric contraction of the muscle was measured by means of a strain-gauge transducer (Nihon Kohden, RM-6000). 'Hypertonic K^+ solution' (60 mM) was prepared by adding an appropriate amount of 2 M KCl solution to the normal medium. When strips of taenia coli were treated with hypertonic 60 mM K^+ medium, the phasic tension was followed by a tonic contraction sustained at a level of $7·34 \pm 0·15$ g ($n = 20$). Addition of 120 mM sorbitol or sucrose, both unable to penetrate the cell membrane of taenia coli (Goodford & Leach 1966), instead of 60 mM KCl had no effect on the contraction, suggesting that the effect of K^+ is not the result of changes in osmolarity.

The stock solution of 0·5 M $MnCl_2$ was prepared, and diluted appropriately, with Tyrode's solution.

Manganese uptake

To determine tissue concentrations of Mn^{2+} in taenia coli, each muscle strip was tied at each end with thread and mounted on a glass rod under a resting tension of 0·6 g. The muscles were then incubated for an appropriate time in different media containing 5 mM Mn^{2+}. The strips were washed successively for 30 min with both Ca^{2+}- and Mg^{2+}-free Tyrode's solution containing 5 mM EDTA, chelating agent which does not penetrate the cell membrane of guinea-pig taenia coli (Brading & Jones 1969). After removal from the bath the strips were blotted on filter paper, then weighed, transferred to a quartz cuvette with 0·5 mL solution containing equal amounts of $HClO_4$ (60%) and HNO_3 (60%) and heated in a muffle furnace at 200°C for 3 h. The samples were dissolved in 0·1 M HCl and Mn^{2+} concentrations were measured by atomic-absorption spectrophotometry (Hitachi, Z-8200).

Statistics

All data are expressed as means ± s.e.m., with the number of measurements given in parentheses. Student's t-test was used to compare data; $P < 0·05$ was considered as indicative of significance.

Results

Effects of nifedipine on the contraction of taenia coli induced by Mn^{2+} in Ca^{2+}-free, K^+ medium

When 5 mM Mn^{2+} was added alone to normal or Ca^{2+}-free medium the muscles of the taenia coli failed to contract even after more than 4 h (data not shown); similar results were obtained for ileal longitudinal muscle (Nasu et al 1994).

The application of 5 mM Mn^{2+} to Ca^{2+}-free, K^+ (60 mM) medium induced initial rapid contraction within 0·5 h followed by gradual redevelopment of tonic tension. The 5 mM Mn^{2+}-induced tension in the taenia coli in Ca^{2+}-free, K^+ medium 3 h after the appearance of the response reached $94·8 \pm 18·7\%$ ($n = 8$) of the original K^+-induced tonic level in normal Ca^{2+} (2·5 mM) medium (Figure 1).

Although low concentrations (10^{-11} or 10^{-10} M) of nifedipine did not affect the initial rapid contraction induced by 5 mM Mn^{2+} in Ca^{2+}-free, K^+ medium, they dose-dependently reduced the later tonic response. Moderate concentrations (10^{-9} or 10^{-8} M) of nifedipine markedly reduced the tonic response, with smaller effects on the initial rapid contraction (Figure 1); nifedipine at a higher concentration (10^{-6} M) completely abolished both responses.

Effects of nifedipine on the contraction of taenia coli induced by Mn^{2+} in the presence of Bay K 8644 in a Ca^{2+}-free medium

In normal Ca^{2+} medium, 10^{-6} M Bay K 8644 alone induced rhythmic contraction which developed into contracture with cessation of the rhythmic contraction $5·6 \pm 0·9$ h ($n = 8$) after application (Figure 2A).

In Ca^{2+}-free medium 10^{-6} M Bay K 8644 induced no response. In Ca^{2+}-free medium, 5 mM Mn^{2+} induced a gradual increase in tension in the

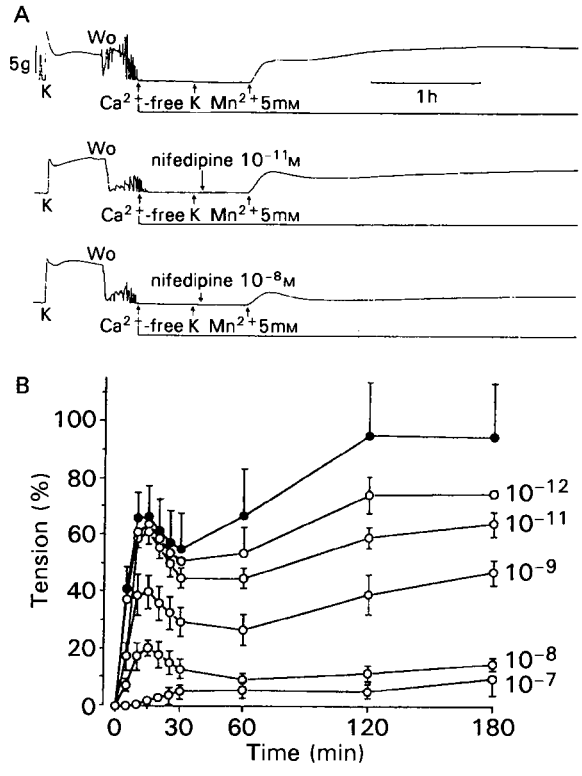

Figure 1. Effects of nifedipine on the contraction induced in taenia coli by Mn^{2+} in Ca^{2+}-free, K^+ medium. A. K^+ (60 mM) was added after suspension for 30 min in Ca^{2+}-free medium with several changes of the solution. Thereafter, 5 mM Mn^{2+} was added after 25 min pre-treatment with nifedipine in the Ca^{2+}-free, K^+ medium. The upper panel shows the control response to 5 mM Mn^{2+} in Ca^{2+}-free, K^+ medium in the absence of nifedipine. K, 60 mM K^+; Wo, Wash out. B. The responses after addition of 5 mM Mn^{2+} to the Ca^{2+}-free, K^+ medium after pre-treatment with nifedipine expressed as percentages of the tonic tension developed 30 min after addition of hypertonic 60 mM K^+ in normal Ca^{2+} (2.5 mM) medium. Each point represents results from eight experiments (mean ± s.e.m.). The accompanying number for each curve (○) indicates the concentration (M) of nifedipine. Curve (●) indicates the contraction induced by Mn^{2+} in Ca^{2+}-free, K^+ medium in the absence of nifedipine.

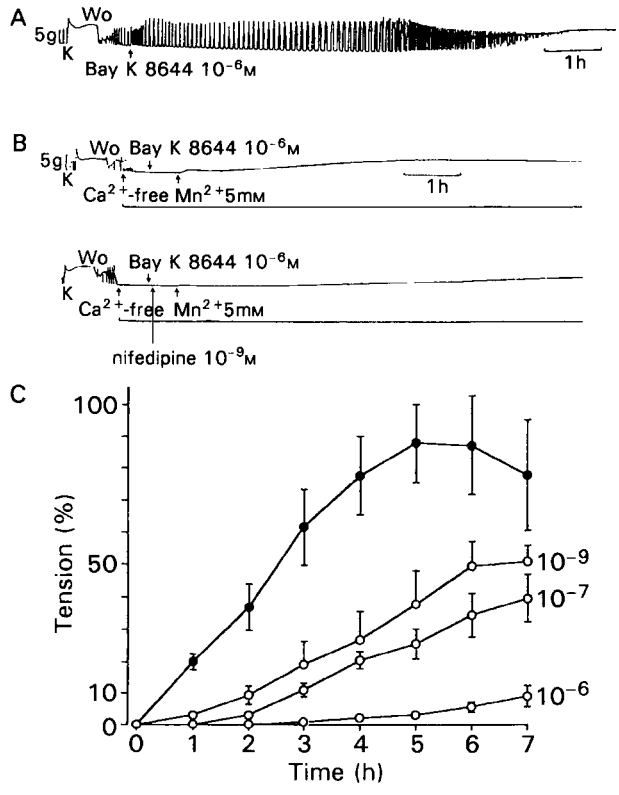

Figure 2. Effects of nifedipine on the contraction induced by Mn^{2+} in the presence of Bay K 8644 in Ca^{2+}-free medium. A. The effect of Bay K 8644 alone on the response of taenia coli in normal Ca^{2+} medium. B. In Ca^{2+}-free medium, Mn^{2+} (5 mM) was added after 25 min pre-treatment with 10^{-9} M nifedipine in the presence of 10^{-6} M Bay K 8644. K, 60 mM K^+. C. The responses after addition of 5 mM Mn^{2+} after pre-treatment with nifedipine in the presence of 10^{-6} M Bay K 8644 expressed as percentages of the tonic tension to K^+ in normal medium. Each point represents results from eight experiments (mean ± s.e.m.). The accompanying number for each curve indicates the concentration (M) of nifedipine. Curve (●) indicates the contraction induced by Mn^{2+} in the presence of Bay K 8644 in Ca^{2+}-free medium in the absence of nifedipine.

presence of 10^{-6} M Bay K 8644. Five hours after application of 5 mM Mn^{2+} in Ca^{2+}-free medium in the presence of 10^{-6} M Bay K 8644, the response reached 87·7 ± 12·1% (n = 8) of the original K^+-induced tonic levels observed in normal Ca^{2+} medium (Figures 2B and 2C).

Nifedipine (10^{-9}–10^{-6} M) delayed the onset of the response to 5 mM Mn^{2+} in the presence of 10^{-6} M Bay K 8644 in the Ca^{2+}-free medium, and dose-dependently reduced the maximum responses to Mn^{2+} (Figures 2B and 2C). Nifedipine at higher concentrations (10^{-5} M) completely abolished the response.

Manganese efflux and uptake by taenia coli in the presence of Bay K 8644 in a Ca^{2+}-free medium

Taenia coli strips were pre-incubated with 5 mM Mn^{2+} in Ca^{2+}-free medium or in Ca^{2+}-free medium containing 10^{-6} M Bay K 8644 for 4 h and were then washed with, respectively, Ca^{2+}-free medium or Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA. The manganese content of the muscles reached equilibrium levels 30 min after washing (Figures 3A and 3B). The manganese content of muscles pre-incubated with 5 mM Mn^{2+} in Ca^{2+}-free, 60 mM K^+ medium for 4 h was 7·81 ± 0·24 (n = 8) mmol kg^{-1} wet weight. The manganese content reached equilibrium levels of 57·1 or 27·3%, respectively, of the original tissue manganese content 30 min after washing with, again respectively, Ca^{2+}-free medium or Ca^{2+}- and Mg^{2+}-free medium containing EDTA. These results indicate levels of intracellular manganese where EDTA cannot reach, i.e. manganese uptake by the cells after treatment with Mn^{2+}.

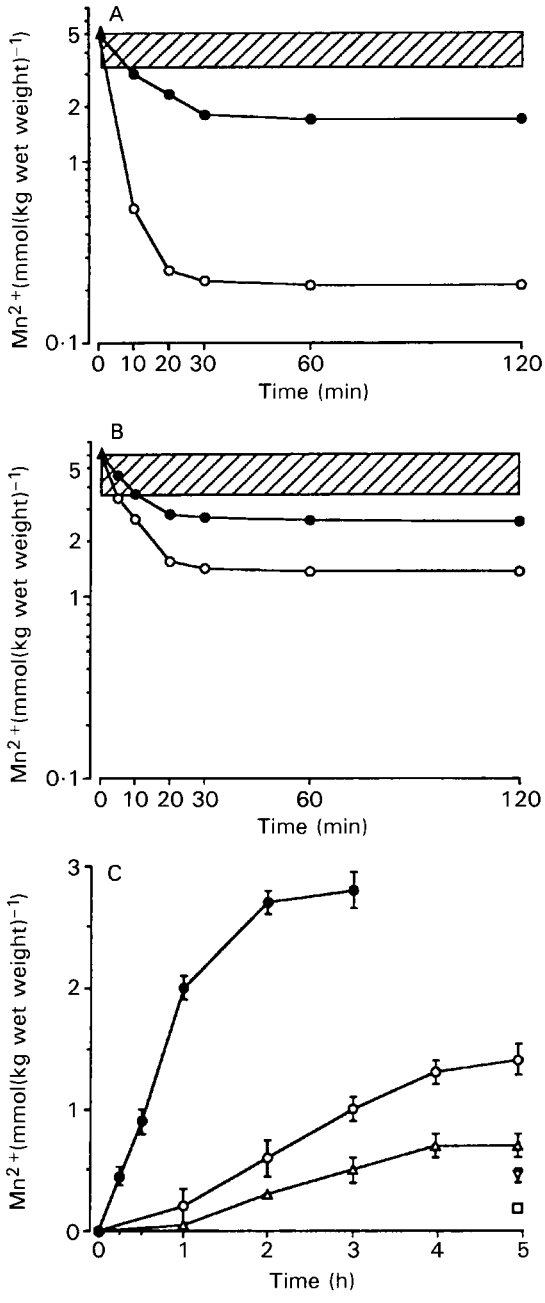

Figure 3. Efflux of manganese from taenia coli. Muscles pre-incubated for 4 h with 5 mM Mn^{2+} in Ca^{2+}-free medium (A) or Ca^{2+}-free medium containing 10^{-6} M Bay K 8644 (B) (▲) were washed with Ca^{2+}-free medium (●) or Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA (○). On the assumption that extracellular space is completely saturated with 5 mM Mn^{2+}, the concentration in the external bathing medium, the hatched area of the figure represents the concentration of Mn^{2+} in the extracellular space (5 mM Mn^{2+} in the external medium $\times 0.35$ in extracellular space measured by [^{14}C]sorbitol (Nasu et al 1983)). Ordinate scale: tissue manganese concentration (mmol kg^{-1} wet weight), logarithmic scale. Each point represents results from eight experiments. The standard errors were less than the size of the symbol. C. Manganese uptake in taenia coli. Mn^{2+} (5 mM) was added to Ca^{2+}-free medium (□) or to Ca^{2+}-free medium containing 60 mM K^+ (●) or 10^{-6} M Bay K 8644 (○) or after 25 min pre-treatment with 10^{-9} (△) or 10^{-7} (▽) M nifedipine in the presence of 10^{-6} M Bay K 8644 in Ca^{2+}-free medium. Each point represents results from eight experiments (mean ± s.e.m.).

To investigate the time-course of manganese uptake by cells of the taenia coli, 5 mM Mn^{2+} was added to Ca^{2+}-free medium or Ca^{2+}-free medium containing 10^{-6} M Bay K 8644 or 60 mM K^+. Bay K 8644 increased manganese uptake by the cells in Ca^{2+}-free medium. The extent of manganese uptake 5 h after addition of Mn^{2+} to Ca^{2+}-free medium in the presence of 10^{-6} M Bay K 8644 was lower than in K^+ medium. Nifedipine (10^{-9} M) inhibited the rate and extent of manganese uptake after addition of 5 mM Mn^{2+} in the presence of 10^{-6} M Bay K 8644 (Figure 3C).

Discussion

^{45}Ca uptake determined by the lanthanum method remained unchanged from control levels after application of 5 mM Mn^{2+} in K^+ medium containing normal Ca (2·5 mM), despite the development of tension in the guinea-pig ileum (Nasu et al 1994). Furthermore, neither ryanodine, an opener of Ca^{2+}-permeable channels in the sarcoplasmic reticulum, nor cyclopiazonic acid, a specific sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, had any effect on the contractions induced by Mn^{2+} in ileum in Ca^{2+}-free, K^+ medium (Nasu et al 1995). These findings suggest that the ileal tension-stimulating action by Mn^{2+} in K^+ medium is unconnected with an increase in Ca^{2+} influx from extracellular fluid or release of Ca^{2+} from storage sites.

When 5 mM Mn^{2+} was added in the absence of Bay K 8644 or K^+, no development of tension was observed, although a very small amount of manganese uptake was observed in the taenia coli in the Ca^{2+}-free medium (Figure 3C). This might indicate that Mn^{2+} enters the restricted cytoplasmic space near the inner surface of the cell membrane through a leaky pathway and that Mn^{2+} entering through such a pathway does not induce contraction. Mn^{2+} has been shown to penetrate the cell membrane through a similar leaky pathway in vascular smooth-muscle cells (Chen & Rembold 1992; Chen & Van Breemen 1993) and through voltage-dependent Ca^{2+} channels.

In this study the manganese content of muscles after addition of 5 mM Mn^{2+} to Ca^{2+}-free medium containing Bay K 8644 for 4 h reached equilibrium levels 30 min after washing with Ca^{2+}-free medium or the same medium containing EDTA. The manganese removed by washing with Ca^{2+}-free medium is considered to be that which exists in the extracellular space and is bound loosely to the cell membrane of the taenia coli. The chelating agent EDTA does not penetrate the cell membrane of guinea-pig taenia coli (Brading & Jones 1969) and

its action is restricted to the cell membrane of smooth muscle (Goodman & Weiss 1971). In addition, EDTA has marked affinity for Mn^{2+} (the logarithm of the equilibrium constant = 14·04; Anderegg 1964) and so the manganese eliminated by action of EDTA would contain metal bound to binding sites such as SH groups on the surface membrane. The manganese fraction which was not eliminated by EDTA might represent manganese which is accumulated in the intracellular compartment which cannot be reached by EDTA. It is thought that EDTA in Ca^{2+}- and Mg^{2+}-free medium will remove Ca^{2+} or Mg^{2+} from binding sites of the cell membrane and that the cell membrane might thereby become permeable to cations. However, in this work the retention of manganese after addition of the metal in the presence of Bay K 8644 was maintained at the equilibrium level after the treatment with EDTA for a longer time (120 min; Figure 3B). This indicates that the facilitating effect of EDTA on manganese loss from the intracellular compartment is small.

Nifedipine dose-dependently reduced both tension development and manganese uptake induced by Mn^{2+} in the presence of Bay K 8644. This indicates that Mn^{2+} can enter the cytoplasm through the dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels activated by Bay K 8644 and then induce contraction.

Mn^{2+} (5 mM) induced a rapid increase in tension (approximately 70% of the maximum tension) 15 min after application in the Ca^{2+}-free, K^+ medium, at which point the intracellular manganese concentration was approximately 15% of the maximum concentration in the K^+ medium. In the presence of Bay K 8644, 5 mM Mn^{2+} induced a gradual increase in tension, and the tension reached the maximum level 5 h after addition of Mn^{2+}. The Mn^{2+}-induced maximum tension in the presence of 10^{-6} M Bay K 8644 was comparable with that in the K^+ medium. However, during the development of maximum Mn^{2+}-induced tension, the concentration of intracellular manganese in the presence of Bay K 8644 was approximately 50% of the maximum concentration in the K^+ medium. It is probable that Mn^{2+} within the cell has some amplifying effect on tension development in the early phase in the presence of K^+ and in the late phase in the presence of Bay K 8644.

In this study low concentrations of nifedipine dose-dependently reduced the tonic response induced by Mn^{2+} in Ca^{2+}-free, K^+ medium with smaller effects on the initial rapid contraction. Karaki et al (1984) reported that in taenia coli, verapamil, a Ca^{2+} antagonist, also preferentially inhibited sustained contraction to K^+ rather than the initial phasic contraction in normal Ca^{2+} medium. Thus, the L-type Ca^{2+} antagonist had a similar effect on the response to Mn^{2+} as that observed in the current study, depending on the Mn^{2+} influx in a Ca^{2+}-free, K^+ medium, and the sustained response to K^+ (Karaki et al 1984) depending on the Ca^{2+} influx in normal Ca^{2+} medium.

In conclusion, Mn^{2+} enters the cytoplasm of guinea-pig taenia coli cells through dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels activated by Bay K 8644, and then induces contraction.

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