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Effect of tetracaine and glibenclamide on $^{45}Ca^{2+}$ handling by isolated pancreatic islets

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1 The $^{45}Ca^{2+}$ uptake in β-cell-rich *ob/ob*-islets was measured using the La^{3+} wash technique.

2 Tetracaine (1 mM) markedly enhanced the $^{45}Ca^{2+}$ net uptake (120 min) in the presence of 3 mM glucose, and at 7 and 20 mM glucose there were clear tendencies to dose-dependent increases with 0.1 to 1 mM tetracaine. Glibenclamide 1 μM to 0.2 mM, stimulated the $^{45}Ca^{2+}$ net uptake in the presence of 3 mM glucose and 0.1 mM to 0.2 mM glibenclamide potentiated the uptake in the presence of 7 mM glucose. When the drugs were added for only a 10 min incubation period, glibenclamide, 1 μM to 0.2 mM, but not tetracaine (10 μM to 1 mM) increased the short-term uptake of $^{45}Ca^{2+}$. After preincubation with either of the drugs, neither tetracaine (10 μM to 1 mM) nor glibenclamide (10 nM to 0.2 mM) had any effect on the short-term $^{45}Ca^{2+}$ uptake.

3 In islets incubated with $^{45}Ca^{2+}$ and tetracaine and washed without La^{3+} the apparent net uptake of $^{45}Ca^{2+}$ was reduced by 0.5 to 1 mM tetracaine both at 3 and 20 mM glucose.

4 Tetracaine (0.5 mM) stimulated the $^{45}Ca^{2+}$ efflux in the presence of 3 mM glucose.

5 The results show that both drugs affected the Ca^{2+} handling. It is suggested that glibenclamide mainly increases Ca^{2+} influx by voltage-dependent pathways, whereas tetracaine, at certain concentrations, mobilizes Ca^{2+} from intracellular stores in the islet cells.

Introduction

Ca^{2+} is an important factor in the stimulus-secretion coupling process in most secretory cells (Rubin, 1970). One of the fundamental pieces of evidence for a relationship between Ca^{2+} uptake and secretion was the finding that drugs like the local anaesthetic tetracaine reduced both acetylcholine-induced $^{45}Ca^{2+}$ uptake and catecholamine secretion in the chromaffin cells of the adrenal gland (Douglas & Kanno, 1967; Rubin *et al.*, 1967; Rubin, 1970). Along these lines, Brisson *et al.* (1971) showed that 0.5 mM tetracaine also inhibited glucose-induced $^{45}Ca^{2+}$ net uptake and insulin release in rat pancreatic islets.

We recently observed that both tetracaine and lidocaine affect insulin secretion in a complex manner (Norlund & Sehlin, 1983). At certain concentrations (0.5 to 1 mM tetracaine or 1 to 5 mM lidocaine), the local anaesthetics induced insulin release and tetracaine (0.1 to 0.5 mM) potentiated the secretion evoked by D-glucose, D-mannose, L-leucine, or glibenclamide. This may be associated with α-adrenoceptor or postreceptor function in the β-cells. The effects of tetracaine were effectively inhibited by adrenaline and the more selective α_2-adrenoceptor agonist, clonidine, and were found to be Ca^{2+}-dependent (Norlund & Sehlin, 1983). Against this background it seemed imperative to investigate the dose-dependence of the tetracaine effects on $^{45}Ca^{2+}$ uptake by islets, to see whether stimulation of secretion by certain concentrations of tetracaine are accompanied by a corresponding change in $^{45}Ca^{2+}$ uptake or efflux. Since tetracaine and the hypoglycaemic sulphonylurea, glibenclamide, have similar effects on the osmotic resistance of β-cells and insulin release (Norlund & Sehlin, 1983; 1984), we have compared the effects of these two drugs on the $^{45}Ca^{2+}$ handling by microdissected β-cell-rich islets from *ob/ob*-mice.

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Methods

Animals and isolation of pancreatic islets

Adult mice, homozygous for the gene *ob*, were taken from a local non-inbred stock (Umeå *ob/ob*-mice). The animals were starved overnight before the pancreatic islets were isolated by microdissection under a stereomicroscope (Hellerström, 1964).

$^{45}Ca^{2+}$ uptake

Batches of five islets were preincubated for 30 min in basal medium. This medium had the following composition (mM): Na^+ 130, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 140, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, HEPES (4-(2-hydroxy-ethyl)-1-piperazineethane-sulphonic acid) 20, D-glucose 3 and bovine serum albumin 1 mg ml^{-1}. The pH was 7.4 and the gas phase was ambient air. The islets were then incubated for 10 or 120 min in 200 µl of the same type of medium labelled with $^{45}Ca^{2+}$ (0.3 TBq mol^{-1}). The incubation was followed by one hour of washing with non-radioactive medium supplemented with 2 mM $LaCl_3$ (Hellman *et al.*, 1976). After freeze-drying and weighing, the islets were dissolved in Hyamine and their radioactivity determined by liquid scintillation counting. Samples of radioactive incubation medium were used as external standards in each experiment.

$^{45}Ca^{2+}$ efflux

The technique used for perifusion of prelabelled islets has been decribed previously (Lindström & Sehlin, 1982). In brief, groups of 30 to 40 microdissected islets were incubated 120 min in basal medium with $^{45}CaCl_2$ (2.9 TBq mol^{-1}). They were then washed for 5 min in non-radioactive basal medium containing 3 mM glucose and transferred to the perifusion chamber. Medium was pumped through the chamber with a peristaltic pump, using a 3 way valve to change medium without interrupting the perifusion. The whole perifusion apparatus was kept in an incubator and maintained at 37°C. The flow rate was 1 ml min^{-1} and control experiments showed that the time required for a new medium to reach the chamber was 30 s. (Correction for this lag period has been made in Figure 4.) After 30 min of perifusion with basal medium, the valve was changed to the same medium containing 3 mM glucose and 0.5 mM tetracaine. Finally, the valve was changed back to the tetracaine-free basal medium and the islets were perifused for another 10 min. Fractions were collected manually over 1 min periods (see legend to Figure 4). After the perifusion, the islets were recovered and the radioactivity of the islets and the effluent fractions were measured. The fractional efflux of $^{45}Ca^{2+}$ was calculated by dividing the content of $^{45}Ca^{2+}$ in each fraction, by the total islet content of $^{45}Ca^{2+}$ at the beginning of that period.

Chemicals

4-(2-Hydroxy-ethyl)-1-piperazineethane-sulphonic acid (HEPES; Boehringer Mannheim GmbH, F.R.G.); bovine serum albumin (Fraction V; Miles Laboratories Inc., Kankakee, IL, U.S.A.); D-glucose and tetracaine (Sigma Chemical Co., St Louis, MO, U.S.A.); glibenclamide (a gift from Svenska Hoechst AB, Stockholm, Sweden); $^{45}CaCl_2$ (Radiochemical Centre, Amersham, U.K.); Hyamine (Packard Co., Downers Grove, IL, U.S.A.). Commercially available reagents of analytical grade and distilled and deionized water were used throughout.

Results

La^{3+}-nondisplaceable $^{45}Ca^{2+}$ uptake

Figure 1 shows the concentration-dependence of the effects of tetracaine on $^{45}Ca^{2+}$ net uptake in islets incubated for 120 min. The La^{3+} wash technique was used to measure specifically intracellular $^{45}Ca^{2+}$ uptake (Hellman *et al.*, 1976). There was a clear

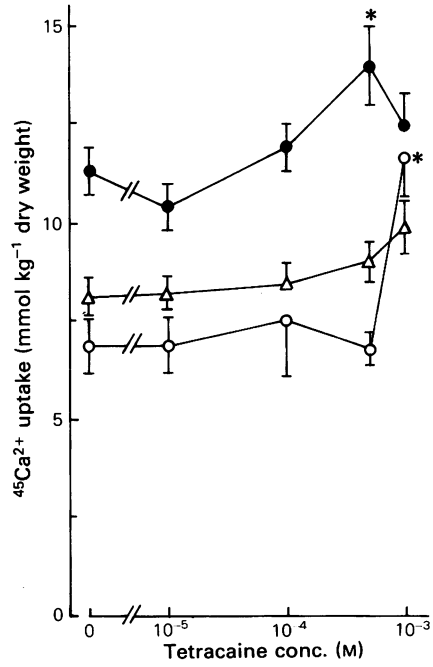

Figure 1 The effects of tetracaine on $^{45}Ca^{2+}$ uptake into pancreatic islets. After a preliminary incubation for 30 min in basal medium, the islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$ and containing 3 mM (○), 7 mM (△) or 20 mM (●) glucose and the various concentrations of tetracaine as indicated. The tetracaine concentrations also apply to the preliminary incubation. The radioactivity retained after washing with lanthanum is expressed in terms of mmol labelled $^{45}Ca^{2+}$ kg^{-1} dry weight of islets. Each point represents the mean, and vertical lines s.e.means from 12–16 separate experiments. $^*P < 0.05$, compared to uptake at 3 mM or 20 mM glucose alone.

tendency for high concentrations of tetracaine to increase $^{45}Ca^{2+}$ uptake in the presence of 3, 7 and 20 mM glucose. At 3 mM glucose, 1 mM tetracaine markedly enhanced the $^{45}Ca^{2+}$ uptake, whereas 10 μM to 0.5 mM of the drug had no effect. At 7 mM glucose, there was a slight, dose-dependent increase by 0.1 to 1 mM tetracaine but none of these groups was significantly different from 3 mM glucose alone. The $^{45}Ca^{2+}$ uptake stimulated by 20 mM glucose was significantly potentiated by 0.5 mM tetracaine, whereas 10 μM, 0.1 mM or 1 mM tetracaine had no such effect. In similar experiments with 120 min incubation 1 μM to 0.2 mM glibenclamide enhanced the $^{45}Ca^{2+}$ uptake at 3 mM glucose and 0.1 μM to 0.2 mM glibenclamide potentiated the effect of 7 mM glucose (data not illustrated).

After preincubation for 30 min with either of the drugs (Figure 2c and d), neither tetracaine (10 μM to 1 mM) nor glibenclamide (1 μM to 0.2 mM) had any effect on the short-term $^{45}Ca^{2+}$ uptake (10 min, apparent initial uptake; Hellman *et al.*, 1976) in the presence of 3 mM glucose. In similar experiments with 7 mM glucose, 10 μM to 1 mM tetracaine or 10 nM to 1 μM glibenclamide had no effect (data not illustrated). For comparison, in similar experiments 20 mM glucose increased the short-term uptake of $^{45}Ca^{2+}$ by 124% from 1.92 ± 0.41 (3 mM glucose) to 4.30 ± 0.79 ($P < 0.02$; $n = 6$).

Figure 2 also shows the results from experiments where tetracaine or glibenclamide were added only during the 10 min incubation with $^{45}Ca^{2+}$. Tetracaine (Figure 2a) had no statistically significant effect on the $^{45}Ca^{2+}$ uptake in the presence of 3 mM glucose, nor did it markedly affect the glucose-induced $^{45}Ca^{2+}$ uptake. The increase in $^{45}Ca^{2+}$ uptake induced by 20 mM glucose, as expressed in terms of mmol $^{45}Ca^{2+}$ kg^{-1} dry islet ($n = 12$) were; without tetracaine (control), 1.09 ± 0.25; plus 10 μM tetracaine, 1.35 ± 0.23; plus 0.1 mM tetracaine, 1.22 ± 0.21; plus 0.5 mM tetracaine, 0.76 ± 0.24; and plus 1 mM tetracaine, 0.70 ± 0.29 ($P < 0.05$ for all glucose-induced increments; 0.70 is not significantly different from 1.09). Glibenclamide, 1 μM to 0.2 mM added only during the 10 min incubation, increased the short-term uptake of $^{45}Ca^{2+}$ in the presence of 3 mM glucose (Figure 2b). Apparently, the maximum effect of glibenclamide was reached at 1 μM.

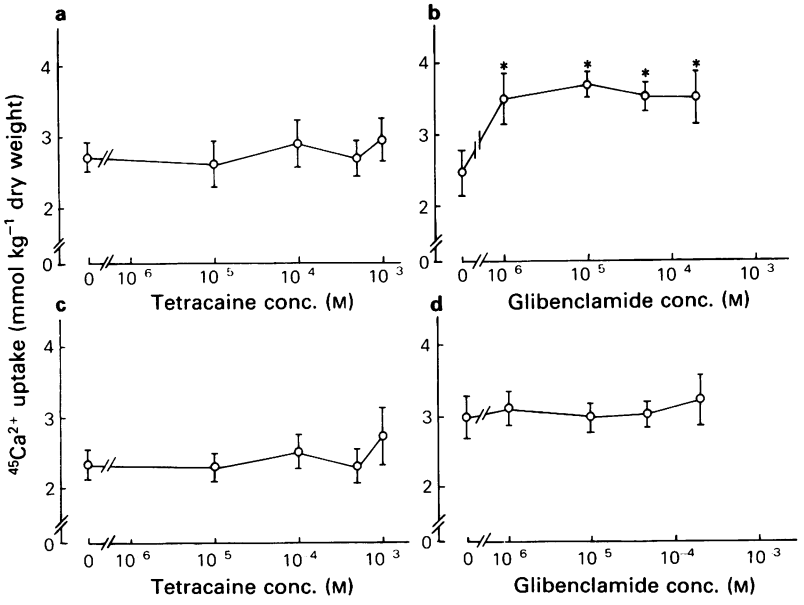

Figure 2 Effects of tetracaine and glibenclamide on short-term uptake of $^{45}Ca^{2+}$ into pancreatic islets. Islets were incubated for 10 min in medium labelled with $^{45}Ca^{2+}$ containing 3 mM (O) glucose and various concentrations of tetracaine or glibenclamide as indicated. Experiments where the islets were initially preincubated for 30 min in basal medium without test substances (a and b). Similar experiments where the islets were initially preincubated for 30 min in basal medium with the same concentrations of test substances as given on the abscissae (c and d). The radioactivity is expressed as in Figure 1. Each point represents the mean, and vertical lines s.e.means, from 8–12 separate experiments. *$P < 0.05$, compared to uptake at 3 mM glucose alone.

$^{45}Ca^{2+}$ uptake measured without La^{3+}

Early experiments by Brisson *et al.* (1971) showed that tetracaine inhibits glucose-induced insulin release and $^{45}Ca^{2+}$ net uptake in rat isolated islets. To simulate the experimental design used by these authors, we performed a set of experiments using 30 min of washing without La^{3+}. Figure 3 shows the retention of $^{45}Ca^{2+}$ in islets first incubated for 120 min with 3 or 20 mM glucose and with different concentrations of tetracaine and then washed for 30 min in basal medium. Clearly, 0.5 to 1 mM tetracaine abolished the glucose-stimulated $^{45}Ca^{2+}$ uptake when measured using this technique. Also the retention time of $^{45}Ca^{2+}$ taken up at 3 mM glucose was slightly reduced by tetracaine.

$^{45}Ca^{2+}$ efflux

The dynamics of the $^{45}Ca^{2+}$ efflux are shown in Figure 4. With 3 mM glucose alone, the mean fractional efflux rate measured from 25–30 min was $0.0164 \pm 0.0018\ min^{-1}$ ($n = 7$) and it slowly decreased approximately linearly with time with a half-life of about 40 min. When 0.5 mM tetracaine was added, the efflux rate started to rise after 2 min and within 6 min it reached the maximum value. After withdrawal of tetracaine from the perifusate, the stimulated efflux proceeded almost unchanged for at least another 10 min.

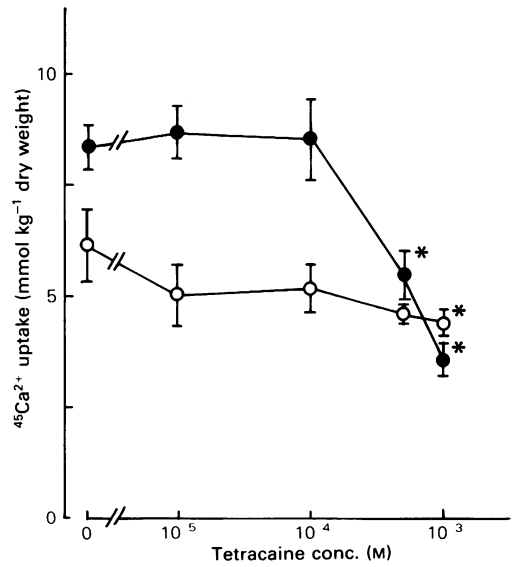

Figure 3 Effect of washing with a medium devoid of La^{3+} on the effects of tetracaine on $^{45}Ca^{2+}$ uptake. After a preincubation as in Figure 1, islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$ containing 3 mM (○) or 20 mM (●) glucose and various concentrations of tetracaine. The radioactivity retained after washing for 30 min at 37°C in basal medium is expressed in terms of mmol labelled $^{45}Ca^{2+}$ kg^{-1} dry weight of islets. Each point represents the mean, and vertical lines, s.e.means, from 8–12 separate experiments. *$P < 0.05$ compared to effects of 3 mM or 20 mM glucose alone.

Discussion

Various secretagogues (Hellman *et al.*, 1977) including glibenclamide (Malaisse *et al.*, 1971) have been shown to increase the $^{45}Ca^{2+}$ net uptake. This effect is likely to be due to an increased influx of calcium, as illustrated here by the increase in uptake of $^{45}Ca^{2+}$ during the initial 10 min (apparent initial uptake; Hellman *et al.*, 1976). Interestingly enough, the stimulatory effect on the 10 min-uptake was abolished by 30 min of prein-

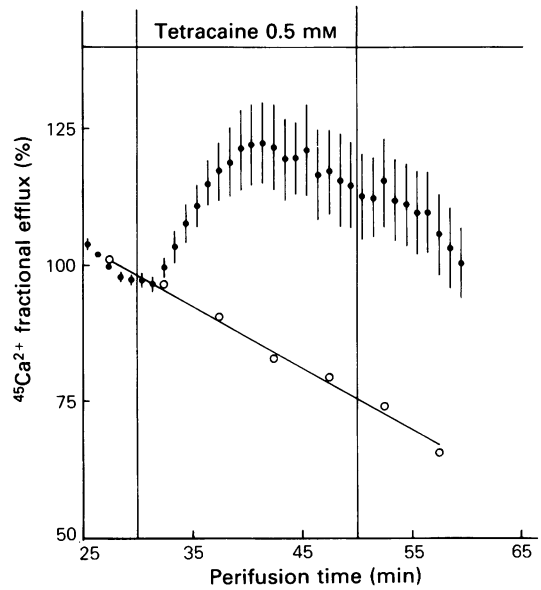

Figure 4 The effect of tetracaine on the efflux of $^{45}Ca^{2+}$ from pancreatic islets. Microdissected islets were labelled for 120 min with $^{45}Ca^{2+}$. They were then placed in a perifusion chamber and perifused with non-radioactive medium with additives as indicated. Data are presented as mean values from 2 control experiments for the efflux at 3 mM glucose throughout (○) and mean values, with vertical lines showing s.e.means, from 5 experiments with 0.5 mM tetracaine (●). The results are calculated as fractional efflux (min^{-1}) and expressed in relation to the mean value, measured from 25–30 min, in each individual experiment ($0.0164 \pm 0.0018\ min^{-1}$; mean ± s.e.mean from 7 experiments).

cubation with glibenclamide. In principle, the same phenomenon has been observed with another sulphonylurea, tolbutamide (Henquin, 1980). The most likely explanation, as suggested for tolbutamide (Henquin, 1980), is that glibenclamide directly or indirectly activates β-cell calcium channels, which are spontaneously inactivated in spite of the continuous presence of the drug.

The stimulating or potentiating effects of tetracaine on insulin release were inhibited by Ca^{2+}-deficiency or the addition of adrenaline or clonidine, suggesting that these effects represent physiologically controlled secretion that may be Ca^{2+}-dependent (Norlund & Sehlin, 1983). However, in a previous study it was shown that tetracaine inhibits glucose-stimulated $^{45}Ca^{2+}$ net uptake (Brisson *et al.*, 1971). To solve this apparent conflict, we performed an extensive study of the dose-dependence of the effects of tetracaine on $^{45}Ca^{2+}$ net uptake, using La^{3+} washing (Hellman *et al.*, 1976) to measure intracellular $^{45}Ca^{2+}$. Clearly, there was no tendency of tetracaine to reduce the $^{45}Ca^{2+}$ net uptake. Instead, 1 mM tetracaine strongly increased the uptake in the presence of 3 mM glucose and 0.5 mM tetracaine potentiated the effect of 20 mM glucose. The dose-dependence of these stimulatory effects is similar to that on insulin release (Norlund & Sehlin, 1983). The increased net uptake was probably not the result of heightened influx of $^{45}Ca^{2+}$ across the β-cell membranes, because tetracaine did not significantly affect the 10 min uptake of $^{45}Ca^{2+}$, whether the islets were pretreated with the drug for 30 min or not.

Thus, although our data on insulin release and $^{45}Ca^{2+}$ uptake are coherent, they are not consistent with the previous findings of inhibited $^{45}Ca^{2+}$ uptake (Brisson *et al.*, 1971). To test whether differences in experimental design were responsible for this discrepancy, we performed a set of experiments designed similarly to those of Brisson *et al.* (1971), using a washing procedure without La^{3+}. With this experimental design, tetracaine at higher concentrations (0.5 to 1 mM) reduced the $^{45}Ca^{2+}$ content of islets incubated with either 20 mM or 3 mM glucose. The key to the difference in results is probably to be found in the site of action of tetracaine. Our measurements of $^{45}Ca^{2+}$ efflux indicate that 0.5 mM tetracaine markedly increased the fractional efflux rate, whereas the La^{3+}-nondisplaceable $^{45}Ca^{2+}$ uptake was not increased at this concentration of the drug. Therefore, it is unlikely that the increased isotope efflux is the result of exchange between cellular $^{45}Ca^{2+}$ and unlabelled Ca^{2+} taken up (Gylfe & Hellman, 1978). Recent experiments have shown that tetracaine (1 mM) also increases the rate of $^{45}Ca^{2+}$ efflux in the absence of extracellular Ca^{2+} (B. Hellman, personal communication). The most probable explanation for this is that tetracaine increases the mobility of Ca^{2+} in intracellular stores in the islets. Since the role of La^{3+} is to block the leakage of $^{45}Ca^{2+}$ from the labelled cells during the washing period, a La^{3+} wash would mask an increased mobility of intracellular $^{45}Ca^{2+}$, whereas washing without La^{3+} would result in a reduced retention. Increased mobility of Ca^{2+} may also explain the increase in net uptake of $^{45}Ca^{2+}$ in experiments with La^{3+} washing.

It has been shown that local anaesthetics, including tetracaine, can produce contractions in striated muscle (Bianchi & Bolton, 1967). One plausible explanation is that these drugs cause a displacement of Ca^{2+} from the sarcoplasmic reticulum or other internal structures (Feinstein & Paimre, 1969). Ca^{2+} is accumulated by internal structures in the β-cells (Howell *et al.*, 1975; Sehlin, 1976; Gylfe & Hellman, 1978; Colca *et al.*, 1982) and it has been suggested that certain drugs, for example methylxanthines, can stimulate insulin secretion by mobilizing Ca^{2+} from such loci (Malaisse-Lagae & Malaisse, 1971; Malaisse *et al.*, 1972; Sehlin, 1976). Against this background, it is tempting to speculate that the stimulating effects of tetracaine on insulin release (Norlund & Sehlin, 1983) are, at least partly, mediated by Ca^{2+} mobilization from intracellular stores.

In conclusion, we suggest that both glibenclamide and tetracaine affect Ca^{2+} handling by the islet cells but via partly different mechanisms. Glibenclamide seems to stimulate Ca^{2+} influx into the cells mainly by affecting the permeability of Ca^{2+}, characterized by a rapid spontaneous inactivation, but may also affect intracellular Ca^{2+} handling. Tetracaine, at certain concentrations, increases the mobility of Ca^{2+} in the cells but has little or no effect on Ca^{2+} influx into the cells. Both actions may lead to increased intracellular Ca^{2+} activity and insulin secretion. The different actions of glibenclamide and tetracaine on Ca^{2+} handling is in accordance with the fact that tetracaine potentiates the insulin release induced by glibenclamide (Norlund & Sehlin, 1983).

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