Mechanism of inhibitory action of TMB-8 [8-(*NN*-diethylamino)octyl-3,4,5-trimethoxybenzoate] on aldosterone secretion in adrenal glomerulosa cells

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The mechanism of 8-(*NN*-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8) action was evaluated in isolated adrenal glomerulosa cells. TMB-8 inhibits both angiotensin II- and K^+ -stimulated aldosterone secretion in a dose-dependent manner. The ID_{50} for angiotensin II- and K^+ -stimulated aldosterone secretion is 46 and 28 μM , respectively. In spite of the fact that 100 μM -TMB-8 inhibits angiotensin II-stimulated aldosterone secretion almost completely, TMB-8 (100 μM) does not inhibit angiotensin II-induced $^{45}Ca^{2+}$ efflux from prelabelled cells nor does it affect inositol 1,4,5-trisphosphate-induced calcium release from non-mitochondrial pool(s) in saponin-permeabilized cells. TMB-8 has no inhibitory effect on A23187-induced aldosterone secretion, but 12-*O*-tetradecanoylphorbol 13-acetate-induced aldosterone secretion is completely abolished. TMB-8 effectively inhibits both angiotensin II- and K^+ -induced increases in calcium influx but has no effect on A23187-induced calcium influx. TMB-8 inhibits the activity of protein kinase C dose-dependently. These results indicate that TMB-8 inhibits aldosterone secretion without inhibiting mobilization of calcium from an intracellular pool. The inhibitory effect of TMB-8 is due largely to an inhibition of plasma membrane calcium influx, but this drug also inhibits the activity of protein kinase C directly.

INTRODUCTION

Malagodi & Chiou (1974) introduced TMB-8 as a non-specific inhibitor of smooth muscle contraction. TMB-8 was shown to inhibit acetylcholine-, nor-adrenaline-, K^+ - and caffeine-induced contraction. In a later study, Chiou & Malagodi (1975) reported that TMB-8 decreases caffeine-induced release of calcium from isolated sarcoplasmic reticulum preparations from skeletal muscle. Since then, this compound has been used frequently as a tool to assess the involvement of intracellular calcium pool(s) in a variety of cellular events (Charo *et al.*, 1976; Smith & Iden, 1979; Matsumoto *et al.*, 1979; Gorman *et al.*, 1979; Rubin *et al.*, 1980; Smith *et al.*, 1980; Juhl *et al.*, 1982; Garcia *et al.*, 1982; Ikeda *et al.*, 1984). The inhibition caused by TMB-8 is sometimes reversed by addition of calcium ionophores, or by elevating the extracellular calcium concentration. In most of the studies, the mode of TMB-8 action was not evaluated directly. The inhibition by TMB-8 was simply interpreted as evidence in support of the involvement of an intracellular calcium pool in cellular response. To date, however, the mechanism of TMB-8 action on the calcium messenger system has not been critically evaluated. It is even unclear whether TMB-8 inhibits agonist-induced mobilization of calcium from an intracellular pool. In spite of the current recognition of TMB-8 as an intracellular calcium antagonist, an inhibitory action of TMB-8 on calcium influx has been described in some systems. Chiou & Malagodi (1975)

showed in an initial study that TMB-8 inhibits resting calcium influx in smooth muscle. Korchak *et al.* (1984) and Sasakawa *et al.* (1984) reported that TMB-8 inhibits agonist-induced calcium influx in neutrophils and adrenal medulla cells, respectively. On the other hand, TMB-8 inhibits the TPA-induced secretory response in neutrophils (Smith & Iden, 1979) and platelets (Show & Lyons, 1982). Since TPA is a potent stimulator of C-kinase (Castagna *et al.*, 1982), Simpson *et al.* (1984) suggested that one target for TMB-8 may be the C-kinase pathway. In addition, a recent report by Brand & Felber (1984) indicates that TMB-8 decreases cellular ATP concentration in thymocytes. Thus, the exact mechanism by which TMB-8 produces its inhibitory action is still unclear, and the mode of its action on the calcium messenger system remains to be determined.

In the present study we have examined the inhibitory action of TMB-8 on angiotensin II- and K^+ -stimulated aldosterone secretion in isolated adrenal glomerulosa cells. In this system, both angiotensin II and K^+ stimulate aldosterone secretion in a calcium-dependent manner (Fakunding *et al.*, 1979; Foster *et al.*, 1981; Schiffrin *et al.*, 1981). Angiotensin II acts by causing a mobilization of calcium from an intracellular pool, and by increasing influx of calcium into cells (Williams *et al.*, 1981; Foster & Rasmussen, 1983; Kojima *et al.*, 1984a, 1985a,b). K^+ acts by stimulating calcium influx via voltage-dependent calcium channels (Foster *et al.*, 1982; Kojima *et al.*, 1984a, 1985b). Results obtained in the present study indicate that: (1) TMB-8 does not inhibit angiotensin

Abbreviations used: TMB-8, 8-(*NN*-diethylamino)octyl-3,4,5-trimethoxybenzoate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; InsP₃, myo-inositol 1,4,5-trisphosphate; KRBG buffer, Krebs–Ringer bicarbonate buffer containing 5.5 mM-glucose.

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II-stimulated calcium release from an intracellular pool; (2) TMB-8 does not inhibit InsP_3 -induced calcium release in permeabilized cells; (3) TMB-8 inhibits both angiotensin II- and K^+ -stimulated calcium influx; and (4) TMB-8 also inhibits the activity of protein kinase C.

MATERIALS AND METHODS

Materials

Angiotensin II, saponin, oligomycin, rotenone, phosphocreatine, creatine phosphokinase, MgATP, histone H1 (IIs), and phosphatidylserine were purchased from Sigma. TMB-8 was from Aldrich. InsP_3 was a gift from Dr. R. F. Irvine (Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.). $^{45}\text{CaCl}_2$ was obtained from Amersham International.

Cell preparations and static incubations

Dispersed calf adrenal glomerulosa cells were prepared by collagenase digestion as described previously (Kojima *et al.*, 1983). Cells were suspended in Krebs-Ringer bicarbonate buffer containing 3.5 mM-potassium, 0.5 mM-calcium and 5.5 mM-glucose (KRBG buffer) unless otherwise mentioned. For calcium influx measurement, 'purified glomerulosa cells' were prepared by using a Percoll density gradient (McNamara *et al.*, 1980). Static incubations were done as described previously (Kojima *et al.*, 1984a).

Perfusion of adrenal glomerulosa cells

Time course of aldosterone secretion and $^{45}\text{Ca}^{2+}$ efflux was studied in a perfusion system using a four-channel flow-through chamber in a Perspex block (Foster & Rasmussen, 1983; Kojima *et al.*, 1985a). Cells (10^7) suspended in Sephadex G-15 were applied to each chamber. The flow rate was 0.4 ml/min and cells were perfused for 40 min before stimulation. Samples for aldosterone were collected every 4 min. Aldosterone was measured by radioimmunoassay using anti-aldosterone antibody provided by the National Hormone and Pituitary Program. When $^{45}\text{Ca}^{2+}$ efflux was measured, cells were incubated with $^{45}\text{CaCl}_2$ (10 $\mu\text{Ci}/\text{ml}$) for 60 min and then applied to the chamber. Samples for $^{45}\text{Ca}^{2+}$ measurement were collected every 2 min. One chamber was perfused without stimulator to obtain fractional efflux of $^{45}\text{Ca}^{2+}$ in the unstimulated control. $^{45}\text{Ca}^{2+}$ efflux is expressed as fractional efflux ratio (Kojima *et al.*, 1985a).

Measurement of calcium influx

'Purified glomerulosa cells' were suspended in KRBG buffer containing 1.25 mM-calcium, 3.5 mM-potassium and 2% bovine serum albumin. Calcium influx was measured by the method of Mauger *et al.* (1984). The calcium uptake was measured at 15, 45, 75 and 105 s and influx rate was calculated from the regression line. Protein was measured by the method of Bradford (1976).

Measurement of calcium release from saponin-permeabilized cells

Saponin-permeabilized cells were prepared as described previously (Kojima *et al.*, 1984b). Cells were suspended in cytosol-like buffer containing 25 mM-Hepes/KOH (pH 7.05), 20 mM-NaCl, 100 mM-KCl, 5 mM-MgSO₄, 0.96 mM-NaH₂PO₄, 1 mM-EGTA, oligomycin (3 $\mu\text{g}/\text{ml}$), rotenone

(0.5 $\mu\text{g}/\text{ml}$), succinate (5 mM), phosphocreatine (5 mM) and creatine phosphokinase (5 units/ml). The concentration of calcium was adjusted to 270 nM with Ca^{2+} -EGTA buffer (Waisman *et al.*, 1981) and $^{45}\text{CaCl}_2$ (1 $\mu\text{Ci}/\text{ml}$). Cells were incubated for various times after addition of 3 mM-MgATP and radioactivity in saponin-treated cells was measured by a filtration method using Whatman GF/C filters.

Measurement of protein kinase C activity

Adrenal glomerulosa cytosol was prepared as described previously (Kojima *et al.*, 1984b). An aliquot of cytosol containing 5 mg of protein was applied to a column (1.0 cm $\times$ 5 cm) of DEAE-cellulose equilibrated with buffer containing 25 mM-Tris/HCl (pH 7.5), 2.5 mM-EGTA, 2.5 mM-MgCl₂, 250 mM-sucrose and 50 mM-2-mercaptoethanol. After adsorption, the column was washed with buffer containing 25 mM-Tris/HCl (pH 7.5), 250 mM-sucrose, 0.1 mM-EGTA and eluted with a linear gradient of NaCl (0–300 mM). Fractions containing C-kinase activity was used as the source of partially purified C-kinase. Protein kinase activity was measured using histone H1 as substrate as described previously (Kojima *et al.*, 1984b).

RESULTS

Effects of TMB-8 on angiotensin II- and K^+ -induced aldosterone secretion

As shown in Fig. 1, TMB-8 inhibits both angiotensin II- and K^+ -induced aldosterone secretion in a dose-dependent manner. At 100 μM , TMB-8 inhibits both angiotensin II- and K^+ -induced aldosterone secretion almost completely. The ID_{50} for angiotensin II-induced secretion is 46 μM , and for K^+ -induced secretion 28 μM . At high doses, TMB-8 also inhibits basal aldosterone secretion. In the following perfusion experiments, 50 and 100 μM -TMB-8 were used to assess the mechanism of TMB-8 action.

Effect of TMB-8 on angiotensin II- and K^+ -induced $^{45}\text{Ca}^{2+}$ efflux and aldosterone secretion

The inhibitory action of TMB-8 was further studied in a perfusion system. As reported previously (Kojima *et al.*, 1985a), angiotensin II causes a rapid efflux of $^{45}\text{Ca}^{2+}$ from prelabeled cells. Following this $^{45}\text{Ca}^{2+}$ efflux response, angiotensin II causes a monotonic and sustained increase in aldosterone secretion (Fig. 2a). In the presence of 50 μM -TMB-8, angiotensin II causes an almost identical initial increase in secretion rate. However, secretory response is only transient; secretion rate is maximum at 20 min and then falls to nearly the basal rate by 48 min. In the presence of 100 μM -TMB-8, angiotensin II causes only a small initial increase, and maximum secretion rate is only 25% of that in the absence of TMB-8. Secretion rate then gradually goes down to a value which is 50% of unstimulated rate. In spite of the great inhibitory effect on aldosterone secretion, TMB-8 (50 or 100 μM) does not inhibit angiotensin II-induced $^{45}\text{Ca}^{2+}$ efflux. TMB-8 does not inhibit angiotensin II-induced $^{45}\text{Ca}^{2+}$ efflux even if it is administered 15 min prior to stimulation (results not shown).

In the absence of TMB-8, K^+ causes a small increase in $^{45}\text{Ca}^{2+}$ efflux and a monotonic increase in aldosterone

Fig. 1. Dose-response curves for TMB-8 action on angiotensin II- and K^+ -stimulated aldosterone secretion

Adrenal glomerulosa cells were incubated for 60 min with various doses of TMB-8 in the presence (○) and absence (●) of 1 nM-angiotensin II (a) or 8 mM- K^+ (b). TMB-8 was added simultaneously with angiotensin II or K^+ . Results are the means $\pm$ S.E.M. for four determinations.

Fig. 2. Time course of effect of TMB-8 on angiotensin II- and K^+ -stimulated $^{45}Ca^{2+}$ efflux and aldosterone secretion

Perfusion was performed to measure $^{45}Ca^{2+}$ efflux from prelabelled cells and aldosterone secretion as described in the Materials and methods section. Cells were stimulated from time 0 to 48 min with 1 nM-angiotensin II (a) or 8 mM- K^+ (b) in the presence and absence of TMB-8. The dose of TMB-8 was 0 (----), 50 μM (○—○) or 100 μM (●—●). Results are the means $\pm$ S.E.M. for three experiments using three different cell preparations.

Fig. 3. Time course of effect of TMB-8 on A23187-, TPA- and TPA + A23187-stimulated aldosterone secretion

Perfusion was performed as described in the Materials and methods section. Cells were stimulated from time 0 to 48 min with 50 nM-A23187 (a), 10 nM-TPA (b) or TPA + A23187 (c) in the presence and absence of TMB-8. The dose of TMB-8 was 0 (○—○), 50 μ M (●—●) or 100 μ M (▲····▲). Results are the means $\pm$ S.E.M. for three experiments using five cell preparations.

secretion rate to a sustained plateau (Fig. 2b). TMB-8 (50 or 100 μ M) completely inhibits the K^+ -induced $^{45}Ca^{2+}$ efflux response. In the presence of 50 μ M-TMB-8, K^+ -stimulated aldosterone secretion is blunted. The maximum secretion rate is approx. 50% of normal. In the presence of 100 μ M-TMB-8, K^+ no longer stimulates aldosterone secretion, and secretion rate goes down slowly. In the absence of stimulator, 50 μ M-TMB-8 does not affect basal secretion rate; however, in the presence of 100 μ M-TMB-8, basal secretion rate goes down rapidly to undetectable levels by 40 min of perfusion (results not shown).

Effect of TMB-8 on A23187-, TPA- and TPA + A23187-induced aldosterone secretion

We have reported that angiotensin II activates two separate intracellular branches of the calcium messenger system, a calcium-calmodulin branch and a C-kinase branch, and that angiotensin II action can be mimicked by stimulating the respective branches using the calcium ionophore A23187 and the phorbol ester TPA (Kojima *et al.*, 1983, 1984b). The effect of TMB-8 on A23187-, TPA- and combined TPA + A23187-stimulated aldosterone secretion was studied with the perfusion system. As shown in Fig. 3(a), TMB-8 (50 μ M) does not inhibit the A23187-induced transient secretion of aldosterone. In sharp contrast, 50 μ M-TMB-8 inhibits TPA-induced secretory response completely. When A23187 and TPA are combined, aldosterone secretory response is similar to that seen after angiotensin II addition. In the presence of 50 μ M-TMB-8, the effect of combined TPA + A23187 is only transient and the resultant secretory pattern is similar to that seen with A23187 alone (Fig. 3c). In the presence of 100 μ M-TMB-8, even A23187-stimulated secretion is greatly inhibited and TPA + A23187-

stimulated secretion is almost identical with that of A23187 alone (Fig. 3c).

Effect of TMB-8 on $InsP_3$ -induced calcium release

Angiotensin II causes a breakdown of phosphatidylinositol 4,5-bisphosphate and generation of $InsP_3$ and diacylglycerol (Farese *et al.*, 1984; Kojima *et al.*, 1984b). $InsP_3$ acts as a second messenger to mobilize calcium from an intracellular pool (Berridge & Irvine, 1984; Kojima *et al.*, 1984b). Therefore, the effect of TMB-8 on $InsP_3$ -induced calcium mobilization was examined in permeabilized cells. When saponin-treated glomerulosa cells are incubated with ATP in the presence of rotenone and oligomycin, these cells accumulate $^{45}Ca^{2+}$ quickly (Fig. 4a). Addition of 1 μ M- $InsP_3$ causes a rapid release of $^{45}Ca^{2+}$ from this pool within 1 min. $InsP_3$ -induced $^{45}Ca^{2+}$ release is not affected by treatment with 50 μ M-TMB-8 (Fig. 4b). Higher doses of TMB-8 up to 100 μ M have no inhibitory effect (results not shown). When TMB-8 is added concomitantly with ATP, permeabilized cells also accumulate $^{45}Ca^{2+}$ quickly. Under this condition, 1 μ M- $InsP_3$ causes $^{45}Ca^{2+}$ release just as in the absence of TMB-8 (results not shown).

Effect of TMB-8 on angiotensin II- and K^+ -induced calcium influx

As shown in Table 1, calcium influx in the presence of TMB-8 (50 μ M) is 80% of basal. Angiotensin II causes a 2.4-fold increase in calcium influx, and this angiotensin II-induced influx is inhibited by 50 μ M-TMB-8. TMB-8 at 50 μ M causes a 40% inhibition of K^+ -induced calcium influx, and a higher dose of TMB-8 has a greater inhibitory effect. TMB-8 (100 μ M) inhibits basal, angiotensin II- and K^+ -induced calcium influx 30%, 50% and

Fig. 4. Effect of TMB-8 on InsP_3 -induced calcium release from permeabilized adrenal glomerulosa cells

Saponin-treated cells were incubated in cytosol-like buffer containing 270 nM free calcium, oligomycin, rotenone, ATP-generating system and $^{45}\text{Ca}^{2+}$ ($10 \mu\text{Ci/ml}$). At time 0, 3 mM-ATP was added. At 20 min, $1 \mu\text{M}$ - InsP_3 (a) or InsP_3 and $50 \mu\text{M}$ -TMB-8 (b) were added. Cells were incubated for the indicated time and the radioactivity in cells was measured by filtration methods. Results are the means $\pm$ S.E.M. for four to six experiments.

Table 1. Effects of TMB-8 on angiotensin II- and K^+ -induced calcium influx

Calcium influx was measured in the presence and absence of TMB-8 as described in the Materials and methods section. Results are the means $\pm$ S.E.M. for five or six experiments using two different cell preparations. Statistical significance was determined by Student's *t*-test. * $P < 0.05$ versus none, ** $P < 0.01$ versus angiotensin II, *** $P < 0.01$ versus K^+ .

Stimulator	Calcium influx (nmol/min per mg of protein)
None	0.56 ± 0.09
TMB-8 ($50 \mu\text{M}$)	$0.44 \pm 0.03^*$
TMB-8 ($100 \mu\text{M}$)	$0.38 \pm 0.04^*$
Angiotensin II (1 nM)	1.35 ± 0.17
+ TMB-8 ($50 \mu\text{M}$)	$0.73 \pm 0.21^{**}$
+ TMB-8 ($100 \mu\text{M}$)	$0.61 \pm 0.20^{**}$
K^+ (8 mM)	2.05 ± 0.18
+ TMB-8 ($50 \mu\text{M}$)	$1.21 \pm 0.24^{***}$
+ TMB-8 ($100 \mu\text{M}$)	$0.76 \pm 0.18^{***}$
A23187 (50 nM)	2.66 ± 0.20
+ TMB-8 ($50 \mu\text{M}$)	2.62 ± 0.32
+ TMB-8 ($100 \mu\text{M}$)	2.51 ± 0.26

60%, respectively. In contrast, A23187-induced calcium influx is not affected by TMB-8.

Effect of TMB-8 on the activity of protein kinase C

Protein kinase C was partially purified from adrenal glomerulosa cells, and the activity of the enzyme was

Table 2. Effect of TMB-8 on the activity of partially purified C-kinase

Partially purified C-kinase was used as an enzyme source as described in the Materials and methods section. The concentration of calcium and phosphatidylserine (PtdSer) were $150 \mu\text{M}$ and $12.5 \mu\text{g/ml}$ respectively. Values are means for three determinations and representative of two separate experiments with similar results.

Addition	Protein kinase activity (c.p.m.)
EGTA	1802
Ca^{2+}	3403
Ca^{2+} + PtdSer	48721
Ca^{2+} + PtdSer + TMB-8 ($50 \mu\text{M}$)	38025
Ca^{2+} + PtdSer + TMB-8 ($100 \mu\text{M}$)	28823
Ca^{2+} + PtdSer + TMB-8 ($250 \mu\text{M}$)	21112

measured using histone H1 as substrate. Addition of calcium and phosphatidylserine increases the activity of protein kinase C. TMB-8 has a dose-dependent inhibitory effect on the activity of this kinase supported by calcium and phosphatidylserine (Table 2).

DISCUSSION

As shown in Fig. 1, TMB-8 inhibits both angiotensin II- and K^+ -stimulated aldosterone secretion in a dose-dependent manner. The present study was conducted in an attempt to specify the mechanism of TMB-8 action. Two issues are addressed: whether TMB-8 inhibits agonist-induced mobilization of calcium, and if not, what effects does TMB-8 have on cellular calcium handling?

The results shown in Fig. 2(a) indicate that $100 \mu\text{M}$ -TMB-8 does not inhibit angiotensin II-induced mobilization of calcium even though this compound inhibits angiotensin-induced aldosterone secretion almost completely. Hence, the inhibitory action of TMB-8 is not due to a blockade of intracellular calcium release. Since $50 \mu\text{M}$ -TMB-8 does not affect A23187-induced secretion (Fig. 3a), TMB-8, at least at this dose, may have no inhibitory effect on either the activity of steroidogenic enzymes (Schiebinger *et al.*, 1985) or ATP generation (Brand & Felber, 1984). At higher dose, however, it is possible that TMB-8 has such non-specific side effects because $100 \mu\text{M}$ -TMB-8 inhibits A23187-induced aldosterone secretion without inhibiting calcium influx (Table 1). The inability of TMB-8 to block a release of intracellular pool of calcium is further supported by the observations in permeabilized glomerulosa cells (Fig. 4). In permeabilized cells, InsP_3 causes a rapid release of calcium from an ATP-dependent non-mitochondrial pool, presumably endoplasmic reticulum. TMB-8 has no effect on InsP_3 -induced calcium release. These results are in line with the observation by Simpson *et al.* (1984) that TMB-8 has little effect on thrombin-induced increase in cytosolic $[\text{Ca}^{2+}]$ in platelets incubated in calcium-free buffer. These data raise serious doubts about the action of TMB-8 as an 'intracellular calcium antagonist'.

In contrast to its inability to block calcium release, TMB-8 greatly inhibits angiotensin II- and K^+ -stimulated calcium influx as well as basal influx (Table 1). These observations are in agreement with previous reports

showing that TMB-8 inhibits calcium uptake in smooth muscle (Chiou & Malagodi, 1975), neutrophil (Korchak *et al.*, 1984) and adrenal medulla (Sasakawa *et al.*, 1984). In fact, the temporal pattern of angiotensin II-induced aldosterone secretion in the presence of 50 μM -TMB-8 closely resembles that seen in the presence of the calcium channel blocker, nitrendipine. In nitrendipine-treated glomerulosa cells, the initial phase is not affected but sustained phase is completely abolished (Kojima *et al.*, 1985b). Likewise, the inhibition of K^+ -stimulated $^{45}\text{Ca}^{2+}$ efflux response by TMB-8 may be due to inhibition of calcium influx, because K^+ -stimulated $^{45}\text{Ca}^{2+}$ efflux is secondary to an increase in $^{40}\text{Ca}^{2+}$ influx and is inhibited by removal of extracellular calcium (Kojima *et al.*, 1985a). Thus, a major action of TMB-8 on cellular calcium metabolism is to inhibit calcium influx. As shown in Table 1, TMB-8 does not inhibit A23187-induced calcium influx. This observation may explain why the inhibitory effect of TMB-8 can be overcome by adding a calcium ionophore. TMB-8 also blocks TPA-induced aldosterone secretion (Fig. 3b). Simpson *et al.* (1984) suggested that a target for TMB-8 may be the C-kinase pathway by showing that TMB-8 inhibits TPA-induced serotonin secretion from platelet without changing cytosolic $[\text{Ca}^{2+}]$. The results in the present study confirm and extend their conclusion. As shown in Table 2, TMB-8 has a direct inhibitory action on partially purified C-kinase. Although the inhibitory action of TMB-8 is relatively small, TMB-8 completely inhibits TPA-induced aldosterone secretion. This discrepancy can be explained by the fact that TMB-8 also inhibits calcium influx, since the effect of TPA on aldosterone secretion in adrenal glomerulosa cells is dependent on intracellular calcium concentration (Kojima *et al.*, 1983) and calcium influx plays a critical role in the activity of the C-kinase branch (Kojima *et al.*, 1985b).

Recently, Schiebinger *et al.* (1985) reported that TMB-8 inhibits exogenous cyclic AMP-stimulated aldosterone secretion. Based on that observation, they suggested that cyclic AMP might cause a release of calcium from the intracellular pool. However, inhibition by TMB-8 does not support the notion that cyclic AMP mobilizes calcium from an intracellular pool.

In summary, TMB-8 does not inhibit angiotensin II-induced mobilization of calcium from the intracellular pool. It greatly inhibits calcium influx under both basal and stimulated conditions. Furthermore, TMB-8 has a direct inhibitory action on protein kinase C. Other non-specific side effects cannot be ruled out when this drug is used at high concentrations. Hence, the interpretations of much of the published data obtained using TMB-8 need to be reconsidered.

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