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STIMULATION OF RENIN SECRETION BY 8-(*N,N*-DIETHYLAMINO)OCTYL-3,4,5-TRIMETHOXYBENZOATE (TMB-8)

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Abstract—The intracellular calcium antagonist 8-(*N,N*-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8) prevents the release of Ca^{2+} from cell storage sites. The effect of this compound on renin secretion from rat renal cortical slices *in vitro* was investigated. TMB-8 was a potent stimulant of renin secretion within the concentration range 10^{-5} M to 5×10^{-4} M with an optimum concentration of 2×10^{-4} M. TMB-8 overcame the inhibition of renin secretion by angiotensin II, ouabain, 60 mM KCl and A23187. The results add to the existing evidence that Ca^{2+} is a common inhibitory messenger for a number of compounds which affect renin release and suggest a role for intracellular calcium stores in the regulation of juxtaglomerular cell Ca^{2+} levels.

Renin secretion from the renal juxtaglomerular (JG) cells is affected by a diverse group of compounds. Angiotensin II [1, 2], vasopressin [2, 3], ouabain [2, 4], vanadate [2, 5] and membrane depolarising agents [2, 6, 7] inhibit renin release, while dibutyryl cyclic AMP [2], forskolin [8], β-adrenergic agonists [9], calmodulin inhibitors [8, 10, 11] and cyclosporin A [12] stimulate secretion. There is considerable evidence that intracellular Ca^{2+} acts as a common inhibitory messenger in the control of renin secretion [2, 13]. However, the importance of intracellular calcium stores in the control of renin release is not known. The JG cell may be relatively permeable to Ca^{2+} [14], and studies using ionophores which increase Ca^{2+} entry into the cell have failed to produce consistent inhibition of renin secretion [14, 15]. The ability of intracellular sites of calcium storage to absorb and release Ca^{2+} may therefore be of importance in the regulation of Ca^{2+} availability to the JG cell. Angiotensin II and vasopressin, both potent inhibitors of renin secretion, have been suggested to act by stimulation of calcium mobilisation from intracellular stores in the liver [16] and, in the case of angiotensin II, in the adrenal cortex [17]. The intracellular calcium antagonist 8-(*N,N*-diethylamino) octyl - 3, 4, 5 - trimethoxybenzoate (TMB-8) inhibits the release of Ca^{2+} from intracellular calcium stores [18, 19]. The effects of this compound in blocking the intracellular actions of Ca^{2+} have been described in muscle [18], platelets [20], pancreatic islets [21] and adrenal cortical cells [19]. In the study reported here, the effect of TMB-8 on rat renal cortical slice renin release was investigated.

Renal cortical studies are frequently used to study renin secretion, but the technique has certain disadvantages. Slices incubated *in vitro* generally release renin at a rate of five to ten times the *in vivo* basal secretory rate of about 1% of the tissue renin content per hr [22]. This may be because, *in vivo*, haemodynamic factors produce a partial depolarisation of the JG cell membrane, resulting in an increased permeability to calcium and a low basal renin secretory rate [13]. Churchill *et al.* [22] found that, if renal cortical slices were incubated in buffer containing 35 mM KCl, the rate of renin release was reduced to a level resembling the *in vivo* rate. The use of 35 mM KCl may mimic the normal *in vivo* state of partial depolarisation of the JG cell membrane. Incubation of slices in 35 mM KCl has an added advantage of greatly reducing the variation in renin release between slices. In this report, the effects of TMB-8, angiotensin II, ouabain, 60 mM KCl and A23187 on renal cortical slice renin release in the presence of both 4 mM and 35 mM KCl are described.

MATERIALS AND METHODS

Wistar–Furth rats, 6- to 8-weeks-old, were used for all experiments. Rats were killed by decapitation after stunning, and the kidneys were removed and placed in buffer at room temperature. Thin outer slices (0.35 to 0.45 mm thick) were taken from each side of the kidneys, using a razor blade [23]. A pool of slices was prepared from six to twelve rats. The buffer used for both slice preparation and incubation contained 125 mM NaCl, 19 mM $NaHCO_3$, 2.6 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 2 mg/ml glucose, 1 mg/ml bovine albumin, and 4–60 mM KCl as indicated. TMB-8 (Aldrich Chemical Co., Milwaukee, WI, U.S.A.), ouabain (Sigma Chemical Co., St. Louis, MO, U.S.A.) and angiotensin II (Hypertensin, Ciba, Switzerland) were dissolved in buffer. A23187 (Calbiochem, La Jolla, CA, U.S.A.) was dissolved in dimethyl sulphoxide and then diluted with buffer before adding to incubation tubes. The final concentration of dimethyl sulphoxide was 0.02%, and an equivalent amount of the

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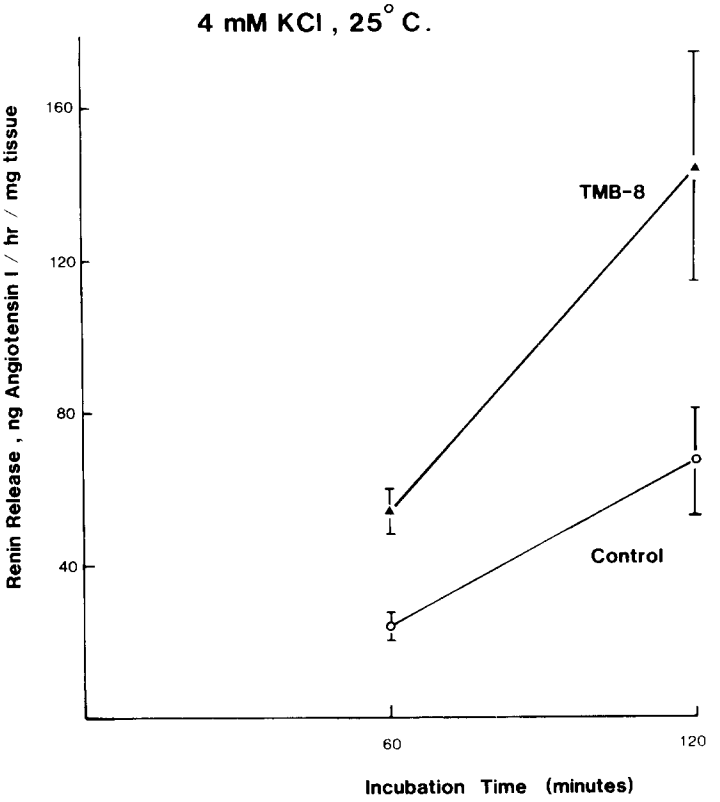

Fig. 1. Effect of TMB-8 (2×10^{-4} M) on renin release from renal cortical slices incubated in buffer containing 4 mM KCl at 25°.

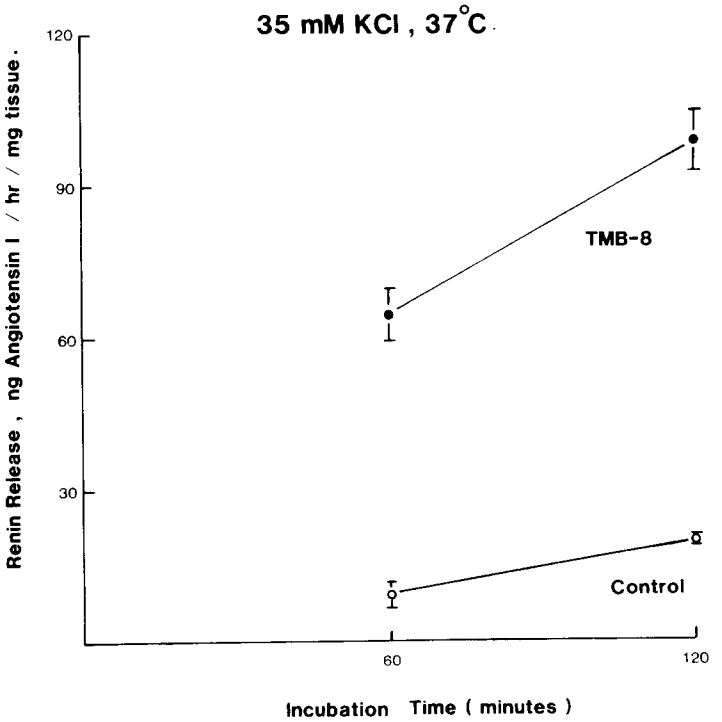

Fig. 2. Effect of TMB-8 (2×10^{-4} M) on renin release from renal cortical slices incubated in buffer containing 35 mM KCl at 37°.

solvent was included in all incubation tubes. Experiments were carried out using either 35 mM KCl at 37° or 4 mM KCl at 25° or 37°. Tubes containing two renal cortical slices in 5 ml buffer were incubated in a shaking water bath under an atmosphere of 95% O_2, 5% CO_2. Aliquots of the incubation medium were taken after 60 and 120 min for renin measurement. All incubations were carried out in duplicate (35 mM KCl experiments) or triplicate (4 mM KCl experiments). After incubation, slices were blotted and weighed.

Renin assay. Renin was assayed in diluted aliquots of the incubation medium by incubation with rat renin substrate preparation. The latter contained 24-hr nephrectomized rat plasma, ethylenediaminetetracetic acid (3×10^{-2} M), phenylmethylsulphonyl fluoride (3×10^{-3} M), and 0.1 M sodium phosphate, pH 7.4. Typically, duplicate 10-μl samples were mixed with 50 μl renin substrate preparation and incubated for 30 min at 37°. The angiotensin I produced was then assayed, using a Clinical Assays Gamma Coat radioimmunoassay kit (Travenol, Cambridge, MA, U.S.A.).

The results are given in each case for one of several experiments in which similar results were obtained. Data from experiments performed on different days were not pooled, because there was some variation in basal renin release between experiments. Results are expressed as mean of replicate slice incubations ± S.E.M. Statistical analysis was performed using Student's *t*-test.

RESULTS

TMB-8 was a potent stimulant of renin secretion throughout the incubation period under all conditions tested. Figures 1 and 2 show the basal and TMB-8-stimulated release of renin over the 2-hr incubation period, using 4 mM KCl at 25° and 35 mM KCl at 37° respectively. A similar effect of TMB-8 was observed using 4 mM KCl at 37° (results not shown). The optimum concentration of TMB-8 was 2×10^{-4} M using both 35 mM KCl (Fig. 3) and 4 mM KCl (results not shown). The stimulation of renin release (Fig. 3) was statistically significant at 10^{-4} M ($P < 0.05$) and 2×10^{-4} M ($P < 0.0025$). At concentrations below 10^{-5} M or above 5×10^{-4} M TMB-8, no stimulation of renin release was found. The apparent inhibition of renin release at 10^{-3} M TMB-8 in the experiment shown in Fig. 3 was not statistically significant ($P > 0.1$). In three similar experiments, there was no significant stimulation or inhibition of renin release by 10^{-3} M TMB-8. The effects of TMB-8 on inhibition of renin release by several compounds are shown in Figs. 4 and 5.

TMB-8 overcame or antagonized the inhibition of angiotensin II (10^{-6} M), ouabain (5×10^{-4} M), and 60 mM KCl at both 4 mM KCl (Fig. 4) and 35 mM

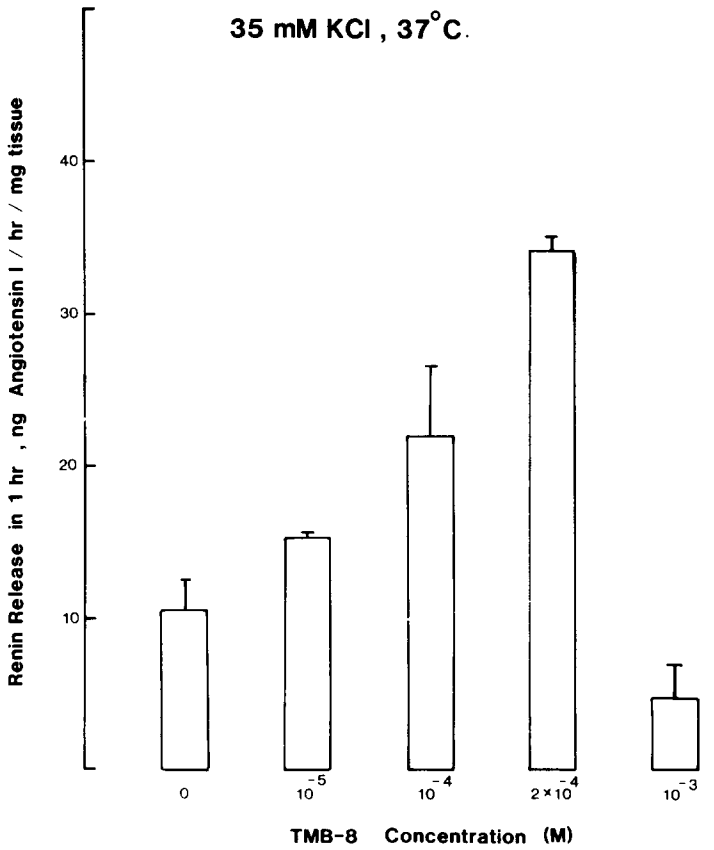

Fig. 3. Effect of TMB-8 concentration on renin release from renal cortical slices in the presence of 35 mM KCl at 37°. Data represent renin released in the 60–120 min period.

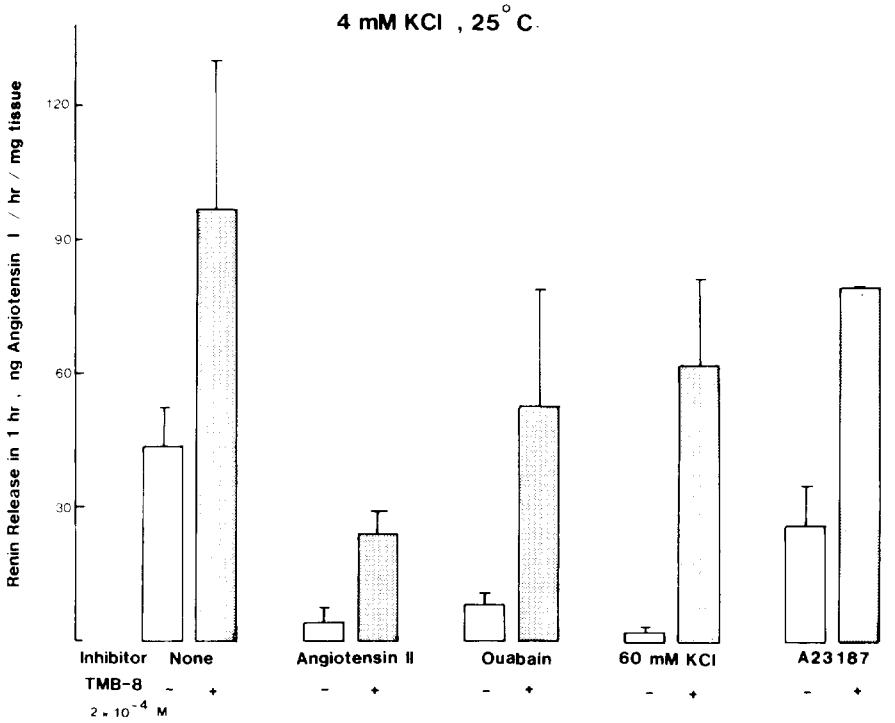

Fig. 4. Effect of TMB-8 (2×10^{-4} M) in the presence of 4 mM KCl at 25° on the inhibition of renal cortical slice renin release by angiotensin II (10^{-6} M), ouabain (5×10^{-4} M), 60 mM KCl and A23187 (10^{-5} M). Data represent renin released in the 60–120 min period.

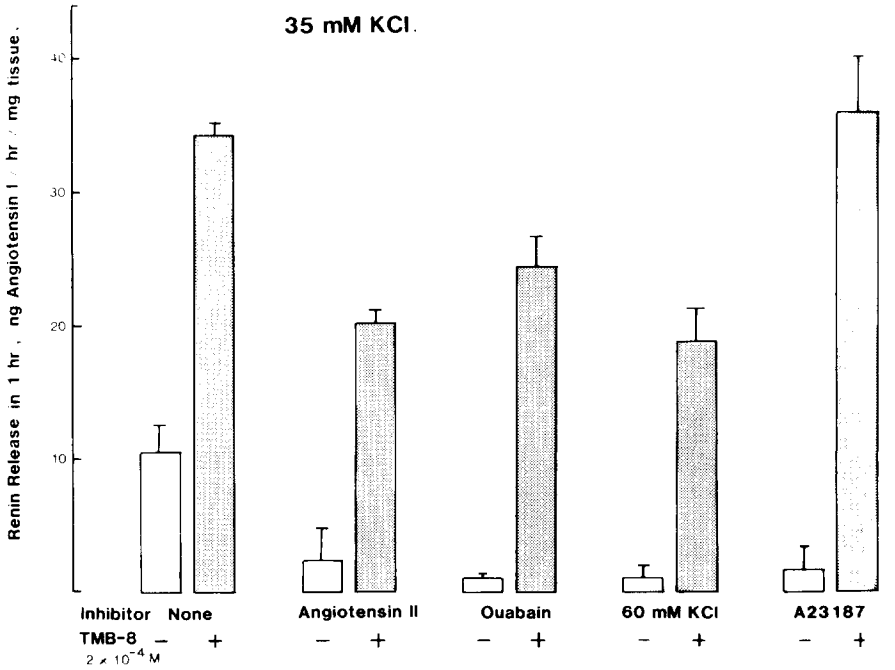

Fig. 5. Effect of TMB-8 (2×10^{-4} M) in the presence of 35 mM KCl at 37° on the inhibition of renal cortical slice renin release by angiotensin II (10^{-6} M), ouabain (5×10^{-4} M), 60 mM KCl and A23187 (10^{-5} M). Data represent renin released in the 60–120 min period.

KCl (Fig. 5). A23187 inhibited renin release at 35 mM KCl and this inhibition was opposed by TMB-8 (Fig. 5). Using 4 mM KCl, A23187 did not consistently inhibit renin release. As shown in Fig. 5 (35 mM KCl), TMB-8 (2×10^{-4} M) stimulated renin release in the presence of inhibitors (compared with control incubations, the statistical significance of this stimulation was $P < 0.05$ for angiotensin II + TMB-8; $P < 0.01$ for ouabain + TMB-8; $P < 0.05$ for 60 mM KCl + TMB-8). The stimulation of renin release by TMB-8 in the presence of all inhibitors except A23187 was significantly less than with TMB-8 alone. In the experiment shown in Fig. 4 (4 mM KCl) where there was a much wider variation between replicates, TMB-8 did not produce a significant stimulation in the presence of the inhibitors (with the exception of A23187).

DISCUSSION

TMB-8 has been shown to inhibit calcium-mediated events such as muscle contraction [18] and hormone secretion [17, 19–21] in a variety of cells and tissues. The mechanism for intracellular calcium antagonism by TMB-8 has been studied using $^{45}Ca^{2+}$ binding experiments [18, 19]. These studies suggest that TMB-8 prevents the release of calcium from intracellular storage sites such as the sarcoplasmic reticulum [18], the mitochondria and the endoplasmic reticulum [19]. Calcium acts as a stimulant for secretion in most secretory cells, but in the JG cell it has an uncharacteristic inhibitory effect on renin secretion [2, 13]. Although it has been suggested that several agents which inhibit renin release do so by promoting Ca^{2+} entry into the cell [2, 13], the mobilisation and sequestration of Ca^{2+} by intracellular calcium stores may also play a role in renin secretion. TMB-8 was shown to be a potent stimulant of renin secretion in the presence of both 4 mM KCl (Fig. 1) and 35 mM KCl (Fig. 2) with an optimum concentration of 2×10^{-4} M (Fig. 3). The loss of the ability to stimulate renin release at 10^{-3} M TMB-8 cannot be fully explained at present. It has been reported that alterations in plasma membrane structure and function occur at this concentration of TMB-8 in platelets [24]. It is possible that similar effects of higher concentrations of TMB-8 in JG cells may prevent the stimulation of renin release.

TMB-8 (2×10^{-4} M) prevented the inhibition of renin release by angiotensin II, ouabain, 60 mM KCl and A23187 (Figs. 4 and 5). Each of these compounds inhibits renin secretion and produces an increase in available intracellular Ca^{2+} by a different mechanism [2, 13, 25, 26]. In muscle [18] and adrenal cells [19], TMB-8 is thought to act by blocking the efflux of Ca^{2+} from storage sites without affecting its influx into stores. The ability of TMB-8 to overcome or antagonize the effects of compounds which increase Ca^{2+} influx into the JG cell suggests that the Ca^{2+} stores in these cells have a large capacity to absorb Ca^{2+}. The stimulation of renin release by TMB-8 alone indicates that the intracellular Ca^{2+} stores are essential for the regulation of intracellular Ca^{2+} levels under basal conditions. In contrast to this result, a recent report [21] showed that TMB-8 has no effect on the basal rate of insulin release from pancreatic islets. As intracellular Ca^{2+} stimulates insulin secretion, the authors concluded that the plasma membrane in these cells was able to regulate Ca^{2+} levels under basal conditions.

If, as has been suggested [14], the JG cell is relatively permeable to Ca^{2+}, the ability of intracellular sites of calcium storage, such as the endoplasmic reticulum, to absorb Ca^{2+} may play a major role in the regulation of cell Ca^{2+} levels and in the control of renin secretion. In some systems, the specificity of TMB-8 as a calcium antagonist has been questioned [24, 27] and other effects of this compound, such as membrane disruption [24] or interference with agonist–receptor binding [27], have been reported. While alternate mechanisms for the stimulation of renin secretion by TMB-8 cannot be ruled out, the antagonism or partial antagonism by TMB-8 of the effects of a diverse group of compounds, each of which alters intracellular calcium levels, suggests a specific calcium-related role for TMB-8.

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