The phorbol ester TPA increases the affinity of exocytosis for calcium in 'leaky' adrenal medullary cells

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Exposure of 'leaky' bovine adrenal medullary cells to the phorbol ester TPA causes a shift in the calcium-activation curve to lower calcium concentrations without altering the levels of secretion at the extremes of the activation curve. These results are consistent with a role for protein kinase C in exocytosis.

Calcium *Exocytosis* *Secretion* *Catecholamine* *Phorbol ester* *Protein kinase C*

1. INTRODUCTION

Many intracellular processes are controlled by local alterations in cytosolic free Ca^{2+} [1,2]. The exocytotic release of catecholamines from bovine adrenal medullary cells is, for instance, activated by a rise in ionized Ca^{2+} from about 100 nM into the μM range [3,4]. In theory, control of Ca^{2+}-dependent processes can also be achieved without such alterations in free Ca^{2+} simply by changing the affinity of the Ca^{2+}-sensitive process for calcium; but rather few examples of this type of control are known at present [5,6]. We now report that the apparent affinity of exocytosis for calcium is increased following exposure of 'leaky' bovine adrenal medullary cells to the phorbol ester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA). This finding is of interest for two reasons:

(i) It illustrates one way by which the rate of exocytosis, a widespread and physiologically important process, can be altered at constant ionized calcium;

(ii) It is consistent with an involvement of protein kinase C [7] in exocytosis.

In the presence of Ca^{2+} and phospholipid, protein kinase C is activated by TPA [8] and this activation, like that of exocytosis, involves a shift in the Ca^{2+}-activation curve to lower Ca^{2+} levels. The activation of this kinase accompanies release of serotonin from platelets [7].

2. MATERIALS AND METHODS

Cells were isolated by protease digestion of thin slices of the bovine adrenal medulla [9] and subsequently rendered 'leaky' to molecules of up to M_r 1000 by exposure to intense electric fields of brief duration [10]. Cells were suspended in a medium containing (mM): K-glutamate, 150; K-Pipes, 20 (pH 6.6); glucose, 5; free Mg, 2; EGTA, 0.5 and various concentrations of Mg^{2+}-ATP. As TPA was dissolved in DMSO, all solutions contained 0.1% (v/v) DMSO.

3. RESULTS AND DISCUSSION

The 'leaky' adrenal medullary cell preparation that is produced after exposure to brief intense fields [10] provides a system in which the environment of the exocytotic apparatus can be subjected to experimental control. Fig.1 illustrates the main features of this preparation and its sensitivity to the phorbol ester, TPA. Exocytosis is dependent on Mg^{2+}–ATP and, in the absence of added TPA, is half-maximally activated by about 1 μM free Ca^{2+}. TPA increases the apparent affinity for Ca^{2+} from 1.2×10^{-6}–5×10^{-7} M, but has no effect on secretion at very low (10^{-8} M) and high (10^{-5} M) concentrations of calcium. This modulating effect of TPA on the affinity of exocytosis for Ca^{2+} is half-maximal at ~2 nM TPA (fig.2a).

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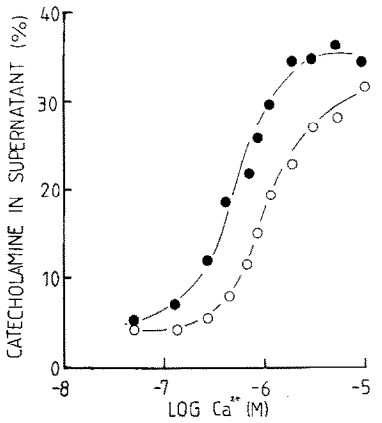

Fig.1. TPA increases the apparent affinity of exocytosis for calcium. Cells in a medium containing 5 mM Mg–ATP were rendered 'leaky' by 10 exposures to 2 kV cm^{-1}, τ 200 μs, and aliquots subsequently challenged with 10 mM Ca^{2+}–EGTA buffers containing either zero (○) or 15 nM (●) TPA. The catecholamine in the supernatant was measured 10 min later and is expressed as a % of the total in the suspension; temp., 23°C.

At intermediate Ca^{2+} concentrations, TPA can still evoke release of catecholamine even when added after secretion has reached a stable level (fig.2b). TPA has no obvious effect on the apparent affinity of the secretory process for Mg–ATP.

It is obviously of interest to determine whether secretion from intact cells is also subject to modulation by TPA. We have examined the effects of exogenous TPA on catecholamine release from intact cells both under resting conditions and in response to acetylcholine or potassium challenges; but have not observed any alteration. Although this may reflect failure of the added TPA to gain access to the cell interior, it might also indicate that the exocytotic process in the intact cell is already operating at the higher Ca^{2+} sensitivity. A higher affinity of exocytosis for Ca in intact cells than is seen in 'leaky' cells might help explain the small quantitative discrepancy between the Ca^{2+} concentration required to effect exocytosis in 'leaky' cells and the size of the Ca^{2+} transient measured with Quin 2 in intact cells [4]. Although TPA seems not to affect intact adrenal cells, it is quite possible that under physiological conditions the apparent affinity of exocytosis may be subject to modulation by agents as yet undefined and, under these circumstances, secretion and membrane turnover

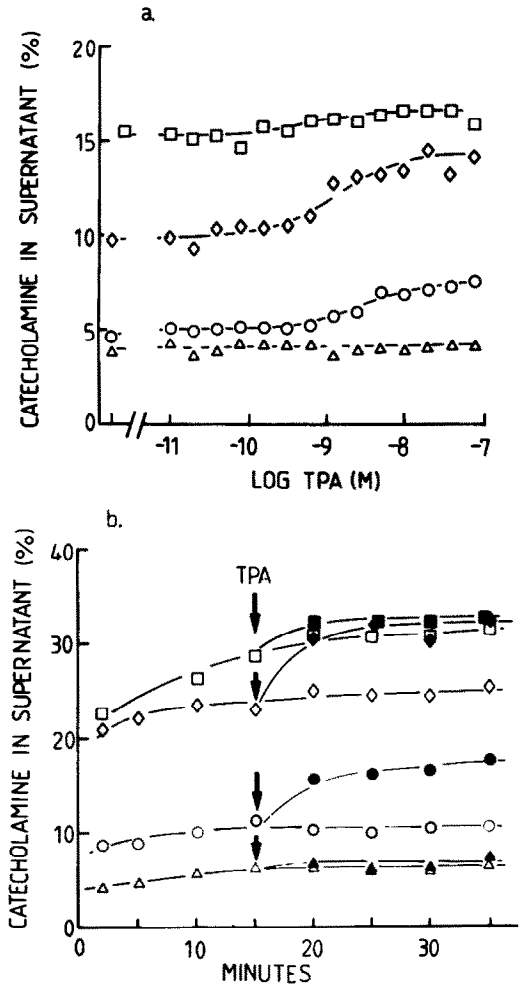

Fig.2. TPA and Ca^{2+}-dependent exocytosis. (a) Dose–response curve for the effect of TPA on catecholamine release at a number of Ca^{2+} concentrations. Cells in a medium containing 1 mM MgATP and EGTA were rendered 'leaky' (10 exposures to 2 kV . cm^{-1}, $\tau = 200\,\mu s$) and aliquots incubated with various concentrations of TPA for 25 min before being challenged with 10 mM Ca^{2+}–EGTA buffers to give approximate free Ca^{2+} concentrations of: 5×10^{-8} M (△); 4.8×10^{-7} M (○); 1.5×10^{-6} M (◇); and 10^{-5} M (□). The catecholamine in the supernatant was assayed to 20 min later; temp., 23°C. (b) Stimulation of exocytosis by TPA at constant ionized Ca^{2+}. Cells in a medium containing 1 mM MgATP and EGTA were rendered 'leaky' as A and aliquots challenged with 10 mM Ca^{2+}–EGTA buffers corresponding to about: 10^{-8} M Ca^{2+} (△); 5×10^{-7} M Ca^{2+} (○); 2×10^{-6} M Ca^{2+} (◇); 10^{-5} M Ca^{2+} (□); 15 min later these cells were further challenged with 0.1% DMSO (open symbols) and 0.1% DMSO containing 15 nM TPA (closed symbols); temp., 23°C.

may be controlled by alterations in either Ca^{2+} or modulator concentrations or a combination of both. The release of serotonin from platelets can be activated by diacylglycerol or TPA without any associated change in cytosolic, free Ca^{2+} [11]. Provided TPA is specific for protein kinase C, these observations and the results described here are consistent with a role for this enzyme in the control of exocytosis.

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