Tumor Promoters Potentiate the Adrenomedullary Secretion Induced by Acetylcholine and Excess K Possibly by Stimulating Phorbol Ester Receptors

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Summary Catecholamine secretion from the perfused cat adrenal in response to 15 mM KCl or acetylcholine was significantly increased by treatment with active tumor promoters. A good correlation was found between potentiation of the secretory response and the phorbol ester receptor binding activity, suggesting that promoters modulate the secretory mechanism via the receptors.

Key words: adrenal medulla, tumor promoter, catecholamines.

There is increasing evidence showing that tumor-promoting phorbol diester 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulates or modulates the release of biologically active substances. For instance, TPA caused a slowly progressing rise in the release of insulin from pancreatic islets (Zawalich et al., 1983), aldosterone from adrenal glomerulosa cells (KoJima et al., 1983), histamine from human leucocytes (Schleimer et al., 1981), and amylase from pancreatic acini (Gunter, 1981). In human platelets, serotonin release by TPA was remarkably increased by the simultaneous addition of a low concentration of Ca ionophore, which is in itself ineffective (YamaniShI et al., 1983). Conversely, release of β-glucuronidase from leucocytes in response to zymosan particles was enhanced by the simultaneous addition of TPA, the secretory effect of which is insignificant (GolDSTEIn et al., 1975).

We now report that some tumor promoters including TPA fail to stimulate catecholamine (CA) secretion from the perfused cat adrenal, but potentiate the response to acetylcholine or high K depolarization possibly via the specific receptors on the plasma membranes.

Adrenal glands of the cats were retrogradely perfused at room temperature (25–28°C) through the adrenolumbar vein (Douglas and Rubin, 1961). Perfusion rate was kept constant (1 ml/min) by means of a peristaltic pump.
modified Locke's medium was used containing NaCl (150 mM), KCl (5 mM), CaCl₂ (2 mM), MgCl₂ (1 mM), Tris/Cl (5 mM, pH 7.0-7.3), and glucose (5.5 mM). Effluents were collected in test tubes containing acetic acid so that final pH was about 4. CA in the effluents was measured by a conventional fluorimetric method (Anton and Sayre, 1962). Phorbol derivatives were dissolved in dimethyl sulfoxide (DMSO), but the final concentration of DMSO never exceeded 0.1 %.

TPA alone (0.16 μM) did not induce CA secretion, but prior exposure to TPA for 15 min increased the secretory responses to 15 mM KCl to 325±43% (N=8) of the preceding responses (Fig. 1), while the second responses in the absence of TPA were 96±4% (N=13) of the first ones. The potentiated responses were markedly reduced by nifedipine (2.5 μM, N=3), a Ca channel blocker, which inhibits the response to 15 mM KCl (Fig. 1).

In order to determine whether the potentiating effect of TPA is related with its tumor promoting activity mediating the particular receptors on the plasma membranes (Shoyab and Todaro, 1980), the effects of some phorbol derivatives on the responses to 15 mM KCl were investigated. Phorbol-12,13-didecanoate (PDD, 1.5 μM), which is an active promoter in mouse skin and inhibits the binding of 3H-phorbol-12,13-dibutyrate (PDBu) (Shoyab and Todaro, 1980), increased the responses to 209±14% of the preceding controls (N=5), but the degree of potentiation was much smaller (122%, mean of two experiments) at a concentration of 0.15 μM than that at the similar concentration of TPA. 4-β-phorbol-13-acetate, which is inactive as a promoter, little influenced the responses (117±16%, N=3, and 105±7%, N=3, at concentrations of 2.5 μM and 0.25 μM, respectively). Similarly, 4-α-phorbol (2.7 μM), which is a non-promoter, did not increase the response at all (100% of controls, mean of two experiments).

We next investigated the effect of teleocidin (using a mixture of teleocidin A and B), since this agent is as active as TPA in many biological actions (Fujiki and Sugimura, 1983) and its promoting activity is reportedly mediated by the TPA

Fig. 1. The potentiating effect of TPA on the secretory response to 15 mM KCl and its inhibition by a Ca channel blocker, nifedipine.
The responses to 15 mM KCl in the presence of teleocidin (0.1 μg/ml) were increased to 249% (mean of two experiments) of the preceding controls. There is thus a good correlation between TPA receptor binding activity and the potentiating action of the secretory response. In fact, our preliminary results showed that the specific binding sites for 3H-PDBu (3.6 nM) are present in the medullary tissues of the cats and its binding activity was markedly inhibited by TPA (100% inhibition at 0.16 μM, N=6) and PDD (96 and 86% inhibition at 1.5 μM and 0.15 μM, respectively. N=2 in each case), but not by 4-α-phorbol (4% inhibition at 2.8 μM, N=2) and 4-β-phorbol-13-acetate (2% inhibition at 2.5 μM, N=2).

The responses to 15 mM KCl in the presence of TPA tended to increase time-dependently (Fig. 1), suggesting the involvement of intracellular reactions in this potentiation. We therefore tested the effects of some agents reported to inhibit some actions of TPA, but 30 min exposure to any agent tested did not block the potentiating effect of TPA on the secretory response. These agents include indo- methacine (5 μM; an inhibitor of prostaglandin synthesis), retinoic acid (1 μM; an antagonist of the tumor promoting action of TPA; Weeks et al., 1979), and palmitoyl carnitine (10 μM; an inhibitor of Ca-dependent, phospholipid-dependent protein kinase; Wise et al., 1982).

TPA also increased the secretory responses to acetylcholine (10 μM) to 239±35% (N=3) of the preceding controls. On the other hand, the responses to histamine (0.5 mM) or Ca (0.5 mM) reintroduction during exposure to a medium lacking divalent cation (Douglas and Rubin, 1963; Sorimachi and Nishimura, 1984) were little affected by TPA (55±11% in case of histamine, N=3, and 52±17% of the preceding controls in case of Ca reintroduction, N=3). These results raise the possibility that TPA somehow activates voltage-dependent Ca channels as does dihydropyridine BAY-K-8644 (Garcia et al., 1984). Even if this is so, however, mode of action of TPA appeared to be different from that of K-8644, since the responses to Ca (0.5 mM) reintroduction were greatly increased in the presence of K-8644 (20±7 times increase, N=3).

In conclusion, our results suggest that some tumor promoters modulate the secretory mechanism possibly via the TPA receptors, although its precise mechanism of action remains to be determined.

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REFERENCES


