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SHORT COMMUNICATIONS

PGE_2, phenylephrine and dopamine-β-hydroxylase release from rat adrenal *in vitro*

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We have recently reported that catecholamine (CA) release from the rat adrenal gland *in vitro* can be inhibited by the addition of α-adrenergic activators and by the prostaglandins PGE_1 and PGE_2 [1, 2]. Catecholamines can be released from adrenergic neurons and from the adrenal medulla by an exocytotic process [3]. It seemed of interest whether α-adrenergic agents and prostaglandins affect the exocytotic mechanism, i.e. the release of dopamine-β-hydroxylase (DBH) together with the catecholamines. Evidence for involvement of exocytosis has been described in the inhibition of catecholamine release from adrenergic nerve-endings: Hedqvist has shown an antagonism by Ca^{2+} of the inhibition of release caused by PGE [4] and Cubeddu *et al.* have recently reported some indirect evidence for the inhibition of DBH release from the nerve endings in the spleen [5].

Adrenal chromaffin cells have no axons and no nerve endings and, therefore, differ in some respects from other adrenergic neurons (e.g. no direct effect of 6-OH-dopamine or of anti-nerve-growth factor serum). The adrenals contain large quantities of CA and, therefore, effects on release of endogenous CA can be studied directly rather than after preloading with labelled CA, which is used in studies on release from adrenergic nerve endings [4, 5]. Therefore, it seemed of interest to study the effect of α-adrenergic activation and of PGE on CA and DBH release from the *in vitro* incubated rat adrenals.

MATERIALS AND METHODS

Male rats of the Hebrew University strain were used throughout; weight 200–250 g. The rats were killed by dislocation of the neck and the adrenals were immediately taken out and placed in 50 ml Ehrlenmeyer flasks containing 10 ml of medium. The medium consisted of NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 0.5 mM; $MgCl_2$, 5 mM; $NaHCO_3$, 1.8 mM; glucose 5 mM.

Incubation and separation of CA. Incubation was carried out in a bath at 37° with constant shaking (100/min). When DBH release was studied bovine serum albumin was added to the medium (final concentration 0.25%). The incubation was stopped after 30 min by immediately placing the flasks in ice, followed by separation of the glands from the medium by centrifugation at 4° 6000 RPM, for 10 min. For CA assay the medium and the glands were acidified and extracted with 0.4 M $HClO_4$.

After centrifugation (to sediment the proteins), the supernatants were brought to pH 5.5 with KOH and centrifuged again. The supernatants were brought to pH 8.4, passed over alumina columns, eluted with 6 ml of 0.05 M $HClO_4$ and then brought to pH 6 and passed immediately through colums of Bio-Rex 70 resin. The CA were finally eluted with boric acid (2/3 M) and assayed with the trihydroxyindole method.

DBH assay. For each determination a batch of glands from 10–14 rats was taken. The glands from control rats

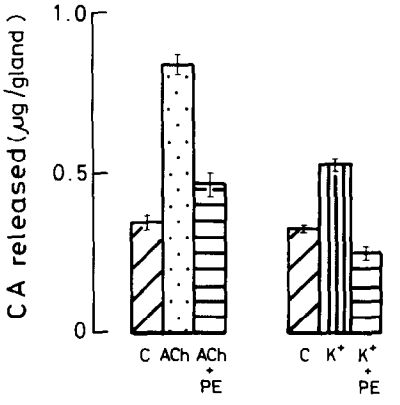

Fig. 1. Effect of phenylephrine on catecholamine release from rat adrenal incubated *in vitro*. C—release in control glands: ACh—release induced by acetylcholine (10^{-4} M); ACh + PE—release induced by acetylcholine (10^{-4} M) in the presence of phenylephrine (10^{-5} M); K^+—release induced by 56 mM K^+ (NaCl concentration was reduced to keep the medium isotonic); K^+ + PE—release induced by 56 mM K^+ in the presence of phenylephrine (10^{-5} M). Each column is the mean of 10 experiments. Vertical bars—S.E.M. Phenylephrine caused a significant reduction of CA release ($P < 0.001$, both in ACh and in high K^+ experiments).

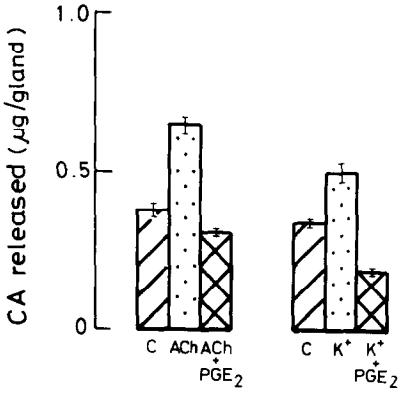

Fig. 2. Effect of PGE_2 on catecholamine release from rat adrenal incubated *in vitro*. C—release in control glands; ACh—release induced by acetylcholine (10^{-4} M); ACh + PGE_2— release induced by acetylcholine (10^{-4} M) in the presence of PGE_2 (1.3×10^{-7} M); K^+—release induced by 56 mM K^+ (NaCl concentration was reduced to keep the medium isotonic); K^+ + PGE_2—release induced by 56 mM K^+ in the presence of PGE_2 (1.3×10^{-7} M). Each column is the mean of 10 experiments. Vertical vars—S.E.M. PGE_2 caused a significant reduction of CA release ($P < 0.001$, both in ACh and in high K^+ experiments).

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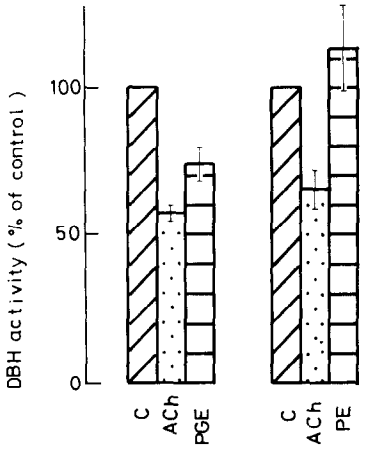

Fig. 3. Effect of PGE_2 and phenylephrine on DBH release from rat adrenal induced by acetylcholine *in vitro*. Ordinate—DBH activity in gland as per cent of activity in control (C) glands. ACh DBH activity in adrenal glands after *in vitro* incubation in the presence of acetylcholine (10^{-4} M); PGE—DBH activity in adrenal gland after *in vitro* incubation in the presence of acetylcholine (10^{-4} M) and PGE_2 (1.3×10^{-7} M); PE—DBH activity in adrenal glands after *in vitro* incubation in the presence of acetylcholine (10^{-4} M) and phenylephrine (10^{-5} M). Each column is the mean of 7 experiments. Vertical bars—S.E.M. PGE_2 reduced the depletion of DBH significantly ($P < 0.05$ compared to ACh alone) but not completely ($P < 0.005$ compared to control). Phenylephrine abolished completely the depletion of DBH caused by acetylcholine ($P < 0.02$ compared to ACh alone, but no significant difference from control (C)).

were taken out and immediately placed in ice. The experimental glands were incubated with or without addition to the medium of the various agents studied. At termination of incubation the glands were separated from the medium. The glands were homogenized in a medium consisting of 0.32 M sucrose and centrifuged at 4° and 1000 *g* for 10 min; the supernatant was collected and centrifuged at 17000 *g* for 60 min. DBH activity was assayed in the sediment, after resuspension in sucrose medium, according to the method of Kirshner *et al.* [6] except for the use of $CuCl_2$ instead of *p*-chloromercurybenzoate to inactivate the inhibitors of DBH.

RESULTS AND DISCUSSION

Figure 1 and Fig. 2 show that CA were released from rat adrenals *in vitro* both by the addition of acetylcholine (ACh, 10^{-4} M) and by increased K^+ concentration in the medium (56 mM). Figure 1 shows that addition of phenylephrine (10^{-5} M), an α-adrenergic activator, inhibited the release of CA significantly. Figure 2 shows that addition of PGE_2 (1.3×10^{-7} M) to the medium also caused a significant inhibition of CA release from the rat adrenal medulla *in vitro*.

The release of CA from the adrenal medulla *in vivo* is mediated through ACh released from the splanchnic nerve and the process of CA secretion is exocytotic [7]. Figure 3 shows that ACh (10^{-4} M) added to the medium of incubation caused a substantial depletion of DBH from the rat adrenals incubated *in vitro*. In one series the depletion was of 43.2 ± 2.4 per cent and in the second series the depletion was 35.0 ± 6.6 per cent of the total activity present initially in the gland. When either phenylephrine (an α-adrenergic activator) or PGE_2 were added simultaneously with ACh the depletion of DBH was significantly decreased. Phenylephrine completely abolished the loss of DBH activity from the glands, whereas PGE_2 reduced the loss from 43.2 to 28.5 per cent.

Thus, both α-adrenergic activation and PGE_2 inhibited an exocytotic process (since they reduced the depletion of DBH caused by ACh). However, PGE_2 may have affected an additional process, too, since the inhibition of DBH release was incomplete, while inhibition of CA release by PGE_2 was complete. Dissociation between inhibition of CA release and of DBH release has also been recently described by Roizen *et al.* [8].

A crucial step in the process of exocytosis is the increase of intracellular Ca^{2+}. It would seem, therefore, that the interference of α-adrenergic activators and (partially) of PGE with the release of CA may involve calcium fluxes. This possibility is now being further investigated.

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