such as during soft-lens desorption the greater quantities of disinfectant may occupy more sites since more molecules are available.

This work was supported by a grant from Burton, Parsons and Co., Inc. and a Public Health Research Grant EY 01413 from the National Eye Institute (K.G.). We thank Mrs Kay Bowman for valuable technical assistance. January 4, 1979

REFERENCES

MacKeen, D. L., Green, K. (1978) J. Pharm. Pharmacol. 30: 678–682

α- and β- Adrenoceptors and PGE_2 in the modulation of catecholamine secretion from bovine adrenal medulla in vitro

P. BOONYAVIROJ, Y. GUTMAN*, *Dept. of Pharmacology, The Hebrew University—Hadassah School of Medicine Jerusalem, Israel*

We have previously reported that release of catecholamines (CA) from rat adrenal medulla incubated in vitro can be inhibited by α-adrenoceptor agonists and by PGE_1 and PGE_2 (Gutman & Boonyaviroj 1975) and enhanced by β-adrenoceptor agonists (Boonyaviroj & Gutman 1977a,b). This is similar to the effect of the same modulators in adrenergic nerve endings (Langer 1974; Starke et al 1977).

However, since rat adrenals incubated in vitro included cortex as well as medulla, a possible mediation or modulation of the observed effects by corticosteroids could not be ruled out, e.g. the α- and β-agonists or PGE_2 could affect primarily the adrenal cortex, and a compound (s) released from the adrenal cortex could then act on the chromaffin cells of the medulla. To clarify this point we have used bovine adrenals, where cortex and medulla can be easily separated.

Bovine adrenals were obtained at the slaughterhouse, immediately placed in ice and the adrenal medulla dissected free of cortical tissue and sliced (10–20 mg per slice). Each slice was placed in a 50 ml Erlenmeyer flask containing 10 ml of Locke solution (mM: NaCl–145, KCl–5·6, $MgCl_2$–5·5, $CaCl_2$–0·5, glucose–5) and incubated at 37 °C for 10 min, with constant shaking. At the end of the incubation, slices were separated from incubation medium and slices and medium were acidified with $HClO_4$. The extracted CA were adsorbed on alumina columns, followed by columns of Biorex 70, as previously described and were assayed (adrenaline and noradrenaline) by the trihydroxyindole method (Feuerstein et al 1977). Catecholamine release is given as a percentage of the total CA present in the slice at the beginning of incubation.

Materials: Acetylcholine chloride and phenylephrine were purchased from Sigma, St. Louis, Mo. Salbutamol was generously supplied by Allen & Hanbury Ltd. Ware, England. Naphazoline was kindly supplied by Assia Ltd. Ramat-Gan, Israel, Phenoxybenzamine was a gift from Smith, Kline & French, Philadelphia, Pa. PGE_2 was kindly sent by Dr J. Pike from Upjohn Co., Inc., Kalamazoo, Mi. H 35/25 ((±)-erythro-4′-methyl-α-(1-isopropylaminoethyl)-benzyl alcohol hydrochloride) was purchased from Kistner Labs. Goteborg, Sweden. Phentolamine was a gift of Ciba, Ltd., Basel.

* Correspondence.

The release of CA into the medium (Locke solution) was 0·25 ± 0·01 μg mg^{-1} of medulla during the incubation. This release constituted 25·1 ± 0·3% of the total CA present in the slice. Fig. 1 shows that acetylcholine (ACh) induced a substantial increase of CA secretion. When α-adrenoceptor agonists, phenylephrine (10^{-5}M) or naphazoline (10^{-5}M), were added, in the presence of ACh (10^{-4}M), the increase of CA secretion induced by ACh was abolished. The addition of the α-adrenoceptor antagonists (phentolamine and phenoxybenzamine) into the medium (without ACh) increased the secretion of CA significantly, compared with the control. Addition of a β_2-adrenoceptor agonist, salbutamol (10^{-6}M) to the medium also enhanced release of CA significantly.

PGE_2 caused a significant inhibition of the release of CA induced by ACh (Fig. 1).

Incubation of medulla in calcium-free Locke solution, supplemented with 2 mM EGTA, caused reduction of

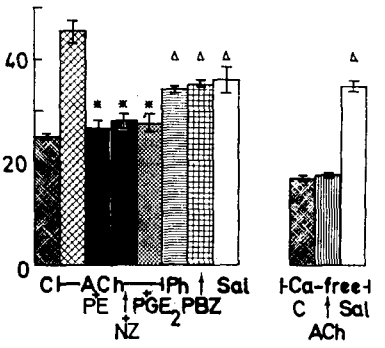

FIG. 1. Effect of various agents on catecholamine secretion (Ordinate: % release) from bovine adrenal medulla slices. C, release of CA during control incubation. ACh, 10^{-4}M acetylcholine present in incubation medium. PE, phenylephrine (10^{-6}M) added to medium. NZ, naphazoline (10^{-5}M) added to medium. PGE_2, PGE_2 (10^{-7}M) added to medium. Ph, phentolamine (10^{-5}M) added to medium. PBZ, phenoxybenzamine (10^{-5}M) added to medium. Sal., salbutamol (10^{-6}M) added to medium. Ca-free, incubation medium without calcium and 2mM EGTA added. Vertical bars, s.e. Each column is the mean of 10 experiments.
*$P < 0.01$ compared with release induced by acetylcholine alone (cross hatched column).
Δ $P < 0.01$—compared with release in control (C).

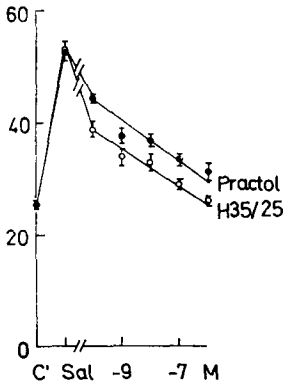

FIG. 2. Effect of β-adrenoceptor antagonists on catecholamine secretion (ordinate: % release) from bovine adrenal medulla slices induced by salbutamol. C' release of CA during control incubation. Sal., release of CA in the presence of 10^{-6}M salbutamol. Abscissa: logarithm of concentration (M) of β-adrenoceptor antagonist added in the presence of 10^{-6}M salbutamol. Practol., practolol: H 35/25 [(±)-erythro-4'-methyl-α-[1-iso-propylaminoethyl] benzylalcorol hydrochloride] a β_2-antagonist. Each point is the mean of 10 experiments. Vertical bars, s.e. Each point on the practolol line differs significantly ($P < 0.05$—$P < 0.01$) from the corresponding point on the H 35/25 line.
[CM].

control CA secretion (compare controls on right and left of Fig. 1). Under these conditions, addition of 10^{-4}M ACh to the medium had no effect on CA secretion. On the other hand, salbutamol was as effective in calcium-free medium as in a calcium-containing medium causing CA secretion.

Fig. 2 shows the effect of β-adrenoceptor antagonists on CA secretion from adrenal medulla, induced by salbutamol. Both practolol, a β_1-antagonist and H 35/25, a β_2-antagonist, caused inhibition of CA secretion. However, the β_2-antagonist was more effective in reducing the CA secretion.

The present report shows that the modulators of CA secretion from adrenal medulla in bovine adrenals act in a similar way to that described for rat adrenals (Gutman & Boonyaviroj 1975, 1977), and for adrenergic nerve endings (Langer 1974; Starke et al 1977). Thus, ACh-induced release of CA is completely dependent on extracellular calcium whereas the β-adrenoceptor stimulation (salbutamol) is independent of extracellular calcium, confirming our previous report (Boonyaviroj & Gutman 1977a). Furthermore, a β_2-antagonist was more effective than a β_1-antagonist (practolol) in reducing CA secretion. This is also in line with reports on β-receptors in adrenergic nerve endings (Stjärne & Brundin 1976).

α-Adrenoceptor agonists inhibited the ACh-induced release of CA, as in rat and human adrenal (Gutman & Boonyaviroj 1975, 1977a,b). The fact that α-adrenoceptor antagonists caused increased CA release would seem to indicate that the CA secreted spontaneously inhibit further release and it is the inhibitory effect of these CA which is abolished by the α-adrenoceptor antagonists.

Finally, PGE_2 inhibited significantly the release of CA, as reported for rat and human adrenal (Gutman & Boonyaviroj 1975; Boonyaviroj & Gutman, 1975, 1977b). The fact that the various modulators—(α-agonists, PGE_2 and β-agonists) were effective in slices of bovine adrenal medulla indicates that the presence of the adrenal cortex is not essential for the action of these agents.

Thus, the present report supports the assumption that the effect of α-agonists and PGE_2 in rat adrenals was due to a direct action on the adrenal medulla. The action of these agents on the medulla of rat, bovine and human adrenals indicates that this effect is of a general nature and is present in adrenals in each species and that adrenomedullary cell membranes resemble in this characteristic the membranes of adrenergic nerve endings. Furthermore, most of the studies on modulation of CA release from adrenergic nerve endings were carried out by first loading the terminals with [^{3}H]-noradrenaline and then following the release of the labelled transmitter. However, the release of ^{3}H-labelled compounds has to be carefully analysed to exclude metabolites. Furthermore, the effect of various agents on overflow of [^{3}H]noradrenaline is, strictly speaking, limited to the pool of newly incorporated CA. In our case, the adrenal medulla has a rich endogenous store of CA and, therefore, the effect of the modulating agents on release of endogenous CA is thus established.

May 2, 1979

REFERENCES

Boonyaviroj, P., Gutman, Y. (1975) Prostaglandins 10: 109–116

Boonyaviroj, P., Gutman, Y. (1977a) Naunyn-Schmiedeberg's Arch. Pharmacol. 297: 241–243

Boonyaviroj, P., Gutman, Y. (1977b) Eur. J. Pharmacol. 41: 73–74

Feuerstein, G., Boonyaviroj, P., Gutman, Y. (1977) Ibid. 44: 131–142

Gutman, Y., Boonyaviroj, P. (1975) Sixth Internat. Cong. Pharmacol. Helsinki, P. 423

Gutman, Y., Boonyaviroj, P. (1977) J. Neural Transm. 40: 245–252

Langer, S. Z. (1974) Biochem. Pharmacol. 23: 1793–1800

Starke, K., Taube, H. D., Borowski, E. (1977) Ibid. 26: 259–270

Stjärne, L., Brundin, J. (1976) Acta. Physiol. Scan. 97: 88–93