Effects of prostaglandin E opposing those of catecholamines on blood pressure and on triglyceride breakdown in adipose tissue

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Vasodepressor and smooth muscle-stimulating activity in seminal plasma and in sheep vesicular gland was first reported by Goldblatt[1, 2] and by von Euler[3, 4]. It was named *prostagladin* by the latter and shown to be attributable to an acidic, lipid-soluble fraction. Bergström and Sjövall[5] isolated in crystalline form from frozen vesicular glands of sheep one active compound, prostaglandin E_1 (PGE_1), the structure of which has recently been shown to be 2(6-carboxyhexyl)-3-(3-hydroxyocten-1-yl)--4-hydroxycylopentanone.[6] Two similar active compounds (PGE_2 and PGE_3) have recently been isolated from the same source.[7] These differ from PGE_1 only by the presence of one and two additional double bonds respectively. PGE_1 has also been isolated from human seminal plasma.[8]

The physiological role of the prostaglandins is not known, although their concentration in the seminal plasma indicates a primary role related to sexual functions as suggested by von Euler.[9] Recently, however, Bergström *et al.*[10] have isolated a biologically active reduction product of PGE_2 in small amounts from lungs of normal sheep and pigs. As all of these related compounds have a very high vasodepressor or smooth muscle-stimulating activity, or both, in man and in experimental animals,[11–13] it is possible that they represent a group of compounds with a more general physiological function. We now wish to report that PGE_1 in microgram doses counteracts both the stimulation of fatty acid release by epinephrine from rat epididymal fat pads *in vitro* and the pressor effect of epinephrine in the intact dog.

The techniques used in the studies of epididymal fat pads have been described[14, 15] and are summarized in the footnotes to Table 1. Effects on blood pressure were studied in mongrel dogs under pentobarbital anesthesia, monitoring mean femoral artery pressure continuously with a Statham strain gauge. Injections of drugs were made through a femoral vein catheter.

The release of glycerol from adipose tissue *in vitro* reflects the rate of lipolysis in the tissue, and this has been shown to be stimulated by epinephrine and several other hormones.[16–19] As shown in Table 1, PGE_1 (pure crystalline compound) in a concentration of 0·1 μg/ml ($2{\cdot}8 \times 10^{-7}$ M) significantly reduced the release of glycerol in the presence of epinephrine, 0·1 μg/ml ($5{\cdot}5 \times 10^{-7}$ M). Concentrations of PGE_1 as low as 0·02 μg/ml ($5{\cdot}6 \times 10^{-8}$ M) had demonstrable effects. Glycerol release in the presence of ACTH and of glucagon was likewise reduced by the addition of PGE_1, 0·1 μg/ml. In experiments similar to those included in Table 1, PGE_1 also decreased release of free fatty acids (FFA) in the presence of norepinephrine, adrenocorticotropic hormone (ACTH), and

Table 1. Effect of PGE_1 on release of glycerol from adipose tissue in the presence of lipolytic hormones*

Hormone and concentration	Glycerol released Without PGE_1	(μmoles/g/hr) Δ Due to PGE_1 †
None	0·9	0·3 ± 0·06
Epinephrine (0·1 μg/ml)	3·1	1·5 ± 0·38
Norepinephrine (0·2 μg/ml)	5·5	2·2 ± 0·13
ACTH (0·04 U/ml)	4·6	1·6 ± 0·21
Glucagon (5 μg/ml)	3·1	1·6 ± 0·27
TSH (10 μg/ml)	3·0	0·3 ± 0·20
TSH (10 μg/ml)	4·7	1·5 ± 0·27‡

* Fat pads incubated 1 hr at 37° in 3 ml Krebs bicarbonate medium containing bovine serum albumin (Armour), 30 mg/ml, and hormone as indicated, one of each pair with and one without PGE_1, 0·1 μg/ml. Six pairs of tissues in each group.

† Mean of differences between paired tissues ± standard error of the mean.

‡ PGE_1, 0·1 μg/ml, added a second time after 30-min incubation.

glucagon. PGE_2 had similar suppressive effects on the FFA release induced by these hormones but was somewhat less potent. Preliminary studies revealed no effect of PGE_2 against the activity of epinephrine.

Unlike the effects of these hormones, which in the amounts used are usually limited to the first 30 min of incubation, the effects of thyroid-stimulating hormone (TSH), 10 μg/ml, persist throughout the hour. Therefore, when a single addition of PGE_1 did not decrease glycerol release in the presence of TSH, PGE_1 was added a second time after 30 min of incubation in another series of experiments. In that group a significant depression of glycerol release was observed.

Recent studies in this laboratory[20] have shown that under appropriate conditions epinephrine (and the other fat-mobilizing hormones listed in Table 1) can effect a 2- to 3-fold increase in lipase activity of the epididymal fat pad. The lipase activity is assayed in whole homogenates of the tissues after incubation with, or in the absence of, the hormone. When PGE_1 is added along with epinephrine it suppresses this hormone-induced activation of lipolytic activity. On the other hand, the activation of adipose tissue phosphorylase brought about by epinephrine[21] does not appear to be influenced by PGE_1.

The effects of PGE_1 in the absence of added hormones were variable. Data from 16 pairs of tissues, however, indicated a significant effect of PGE_1— 0·3 0·06 μmole glycerol/g tissue per hour. Mean glycerol release for the control tissues without hormone was 0·9 μmole/g per hour.

The effect of epinephrine (or norepinephrine), PGE_1, and a combination of the two on mean arterial pressure in two typical experiments are shown in Table 2. In six trials in four dogs the catecholamine alone (2·5 to 5·0 μg/kg) elevated arterial pressure by 76 mm Hg (range 60 to 90); simultaneous injection of PGE_1 (5 to 12·5 μg/kg) reduced the observed pressor response by 52 ± 5·7 per cent ($P < 0.001$). Injection of PGE_1 alone in doses of 6 to 12·5 μg/kg caused a profound drop in blood pressure, in confirmation of previous studies,[11–13] with gradual return to normal over about 15 min.

TABLE 2. EFFECTS OF PGE_1 ON HYPERTENSIVE ACTION OF EPINEPHRINE AND OF NOREPINEPHRINE

	Dose of catecholamine (μg/kg)	Dose of PGE_1 (μg/kg)	Change in mean blood pressure* (mm Hg)
Epinephrine	5	0	+90
	5	12·5	+55
	0	12·5	−45
	2·5	0	+60
	2·5	12·5	+20
Norepinephrine	5	0	+85
	5	5	+50
	5	10	+20
	5	20	−50†

* Increment at peak; reached within 10 to 25 sec. Initial pressures ranged between 100 and 125 mm Hg.

† Transient rise of 20 mm Hg at 15 sec, followed by a progressive fall to 50 mm Hg below control level at 1·5 min and then gradual return to normal at 15 min.

The present studies show that PGE_1 can, operationally, "counteract" the effects of epinephrine and norepinephrine on blood pressure and on fat breakdown in adipose tissue. It should be stressed, however, that PGE_1 alone has potent vasodepressor activity and has some activity on fat breakdown in adipose tissue in the absence of added hormones. Whether its effects are due to a direct antagonism (i.e. interference with the ability of catecholamines to effect a certain sequence of biochemical events) or whether it simply exerts antagonistic pharmacological effects operating through independent mechanisms cannot be stated. It should be noted that Goldblatt obtained evidence for synergism between the action of epinephrine and of a crude alcohol extract of seminal plasma in stimulating

the isolated seminal vesicle of the guinea pig.[2] Still, in view of the present results, the possibility that the intrinsic vasodepressor activity of prostaglandin is related to an effect on endogenous catecholamine action deserves further exploration.

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REFERENCES

1. M. W. GOLDBLATT, *Chem. and Ind.* **52**, 1056 (1933).
2. M. W. GOLDBLATT, *J. Physiol., Lond.* **84**, 208 (1935).
3. U. S. VON EULER, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **175**, 78 (1934).
4. U. S. VON EULER, *Klin. Wschr.* **33**, 1182 (1935).
5. S. BERGSTRÖM and J. SJÖVALL, *Acta chem. scand.* **14**, 1701 (1960).
6. S. BERGSTRÖM, R. RYHAGE, B. SAMUELSSON and J. SJÖVALL, *Acta chem. scand.* **16**, 501 (1962a).
7. S. BERGSTRÖM, F. DRESSLER, R. RYHAGE, B. SAMUELSSON and J. SJÖVALL, *Arkiv Kemi* (1962b). **19**, 563 (1962b).
8. S. BERGSTRÖM and B. SAMUELSSON, *J. biol. Chem.* (1962. **237**, PC3005 (1962).
9. U. S. VON EULER, *J. Physiol. Lond.*, **88**, 213 (1936).
10. S. BERGSTRÖM, F. DRESSLER, L. KRABISCH, R. RYHAGE and J. SJÖVALL, *Arkiv Kemi* (1962c). **20**, 63 (1962c).
11. S. BERGSTRÖM, H. DUNER, U. S. VON EULER, B. PERNOW and J. SJÖVALL, *Acta physiol. scand.* **45**, 145 (1959).
12. S. BERGSTRÖM, R. ELIASSON, U. S. VON EULER and J. SJÖVALL, *Acta physiol. scand.* **45**, 133 (1959).
13. E. ÅNGÅRD and S. BERGSTRÖM, *Acta physiol. scand.* (1962). In press.
14. M. VAUGHAN, *J. biol. Chem.* **236**, 2196 (1961).
15. M. VAUGHAN, *J. biol. Chem.* **237**, 3354 (1962).
16. B. LEBOEUF, R. B. FLINN and G. F. CAHILL, JR., *Proc. Soc. exp. Biol., N.Y.* **102**, 527 (1959).
17. W. S. LYNN, R. M. MACLEOD and R. H. BROWN, *J. biol. Chem.* **235**, 1904 (1960).
18. J. H. HAGEN, *J. biol. Chem.* **236**, 1023 (1961).
19. M. VAUGHAN and D. STEINBERG, *J. Lipid Res.* **4**, 193 (1963).
20. M. VAUGHAN and D. STEINBERG, *Fed. Proc.* **22**, 303 (1963).
21. M. VAUGHAN, *J. biol. Chem.* **235**, 3049 (1960).

Effects of morphine and Tofranil on the incorporation of phosphate (^{32}P) into phospolipids of rat brain slices

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RESULTS of previous work from this laboratory[1, 2] have shown that the addition of a high concentration of potassium ions (105 mEq/L) to, or the omission of calcium ions from, the incubation medium markedly stimulates the incoporation of labeled phosphate (^{32}P) into the phospholipids of slices of cortex from rat brain. In these experiments, the factors influencing the cationic stimulation of ^{32}P incorporation have been found to be similar to those affecting the acetylcholine stimulation of phospholipid labeling. We now find, in confirmation of some earlier results obtained with morphine,[3] that the labeling of phospholipids from inorganic ^{32}P in brain cortex slices is strongly enhanced by the addition of morphine (>2 mM) or by the antidepressant drug, Tofranil (N-(3-dimethylaminopropyl)-iminodibenzyl hydrochloride).