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Role of pre- and postjunctional inhibition by prostaglandin E_2 of lipolysis induced by sympathetic nerve stimulation in dog subcutaneous adipose tissue *in situ*

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Summary

1. Canine subcutaneous adipose tissue was isolated and autoperfused *in situ* after labelling of the noradrenaline stores by ^{3}H-(−)-noradrenaline.

2. Prostaglandin E_2 (10–200 ng/ml) increased blood flow and glucose uptake, and caused a dose-dependent inhibition of lipolysis induced by sympathetic nerve stimulation (4 Hz). The actions of exogenous prostaglandin E_2 are therefore similar to those of prostaglandin E_1 in this tissue. There were no consistent effects of prostaglandin E_2 on the vasoconstriction or on the ^{3}H-noradrenaline overflow induced by nerve stimulation.

3. Phenoxybenzamine (1·5–2 mg i.a.) caused a 5-fold increase in ^{3}H-noradrenaline overflow and a 95% reduction of the vasoconstrictor response to nerve stimulation. The lipolytic response was similar to that of the control. Prostaglandin E_2 (100–200 ng/ml) administered after phenoxybenzamine caused a 90% inhibition of lipolysis, while the vasoconstrictor response was enhanced to about 50% of control. Prostaglandin E_2 inhibited ^{3}H-noradrenaline overflow by about 50% but it was still larger than that of the control.

4. It is suggested that exogenous prostaglandin E_2 inhibits lipolysis induced by sympathetic nerve stimulation mainly by a postjunctional action in canine subcutaneous adipose tissue.

Introduction

Prostaglandins of the E-series are potent inhibitors of catecholamine-induced lipolysis *in vitro* (Steinberg, Vaughan, Nestel & Bergström, 1963), and prostaglandin E_1 has been shown to inhibit the lipolytic effect of sympathetic nerve stimulation in canine subcutaneous adipose tissue *in situ* (Fredholm & Rosell, 1970a). There is evidence that prostaglandin-like material is released upon activation of lipolysis in rat epididymal adipose tissue *in vitro* and in canine subcutaneous adipose tissue *in situ* (Shaw & Ramwell, 1968; Christ & Nugteren, 1970; Fredholm, Rosell & Strandberg, 1970). These facts suggest a physiological role for endogenous prostaglandins as feed back inhibitors of stimulated lipolysis (Bergström, Carlson & Weeks, 1968), a possibility that has recently received experimental support (Illiano & Cuatrecasas, 1971).

Prostaglandins E_1 and E_2 have been shown to inhibit the transmitter release from several sympathetically innervated organs (Hedqvist, 1970, 1972). Prostaglandin E_2 might therefore antagonize both the release of noradrenaline from the

sympathetic nerve endings in adipose tissue (a prejunctional effect) and the lipolytic effect of the released noradrenaline (a postjunctional effect). The aim of the present investigation was to determine if prostaglandin E_2 has effects that are qualitatively and quantitatively similar to those of prostaglandin E_1 especially with regard to the antagonism of lipolysis induced by sympathetic nerve stimulation, and to determine the relative importance of pre- and postjunctional actions of prostaglandin E_2 in producing this inhibition.

Methods

The experiments were conducted on female mongrel dogs weighing 9–18 kg (mean 12·2). Anaesthesia was induced by 25–30 mg/kg i.v. sodium pentobarbitone (Nembutal, Abbott Labs) with further single doses of 25–30 mg i.v. during the course of the experiment, whenever necessary. Arterial blood pressure was measured in the femoral artery by means of a Statham (P 23 AC) pressure transducer. Subcutaneous adipose tissue was isolated from surrounding tissues as described in detail by Rosell (1966). The weight of the tissue varied between 13 and 64 g. After cannulation blood was directed from the ipsilateral femoral artery to the adipose tissue preparation via an exteriorized loop, in which was included a drop-counter to measure blood flow. The venous blood flow was led back to the systemic circulation via the ipsilateral femoral vein. Blood pressure and blood flow were recorded on a Grass Polygraph (Model 4 B).

The nerve to the tissue was cut at the level of the external hiatus of the inguinal canal, placed on a bipolar silver electrode, and protected from drying by Plastobase (Squibb). Pulses of supramaximal intensity (12–13 V) and duration (2 ms) were delivered at the rate of 4 Hz by a Grass stimulator. Although the time of stimulation varied between experiments from 4 to 10 min it was kept constant within each experiment.

Before the start of the experiment, ^{3}H-(−)-noradrenaline (9 Ci/mM, New England Nuclear Corporation, Boston, USA) was infused at the rate of approximately 5×10^{-10} moles per min for 30 min to label the tissue noradrenaline stores. The experiment was not begun until at least one hour after the completion of this infusion.

Arterial and venous blood samples were collected in ice-cooled centrifuge tubes. After determination of the haematocrit (mean: 40%) the blood was centrifuged and aliquots were taken for the determination of glycerol (Laurell & Tibbling, 1966), glucose (Levin & Linde, 1962), and radioactivity (see below). The arterial glucose concentration averaged 7·38 mM and the glycerol concentration 0·113 mM.

The radioactivity of the plasma extracts was separated into that of intact noradrenaline and that of its metabolites by cation exchange column chromatography, as described by Stjärne & Lishajko (1966). The recovery of authentic noradrenaline added to the samples and carried through the entire chromatographic procedure was $85{\cdot}9 \pm 0{\cdot}9\%$ (mean ± S.E.M. $n=6$).

Perfused organs were homogenized in 10 volumes of 0·4 M perchloric acid and the extracts were purified on alumina and analysed fluorimetrically for noradrenaline (Euler & Lishajko, 1961). Correction was made for noradrenaline losses during purification and fluorimetric determination.

Three to four hours after the infusion of approximately 125 μCi of ^{3}H-nor-

adrenaline, 11·8 ($\pm$4·2)$\times 10^4$ dpm remained in the tissue. Intact noradrenaline accounted for 89·5$\times$1·3% of the radioactivity and the specific activity was 3·1 ($\pm$0·7)$\times 10^6$ dpm/μg noradrenaline.

The radioactivity of the different perfusate samples and organ extracts was determined by counting 0·1–1·0 ml aliquots in 10 ml of Insta-Gel (Packard Instr., Co.) in a Packard Liquid Scintillation Spectrometer. All values were corrected for counting efficiency and expressed as dpm.

Prostaglandin E_2 was generously supplied by Dr J. Pike, Upjohn Company, Kalamazoo, USA, and phenoxybenzamine (Dibenzyline) by Smith, Kline & French, Welwyn Garden City, England. Prostaglandin E_2 was dissolved in a minimal amount of ethanol and was diluted with 0·9% w/v NaCl solution immediately before use. The concentration of prostaglandin E_2 in the blood perfusing the adipose tissue was calculated from the measured blood flow and from the infusion rate.

Results

Effect of nerve stimulation

The effects of sympathetic nerve stimulation (4 Hz) on blood flow, glycerol release, ^{3}H-noradrenaline and ^{3}H-normetanephrine overflow in canine subcutaneous adipose tissue are illustrated in Fig. 1 and Tables 1 and 2. These changes are similar to those described earlier in the same tissue by the use of a constant flow technique (Fredholm & Rosell, 1968, 1970b). During stimulation there was little or no change in the overflow of ^{3}H-radioactivity, but there was a marked increase in the overflow of ^{3}H-noradrenaline which accounted for about 40% of the total

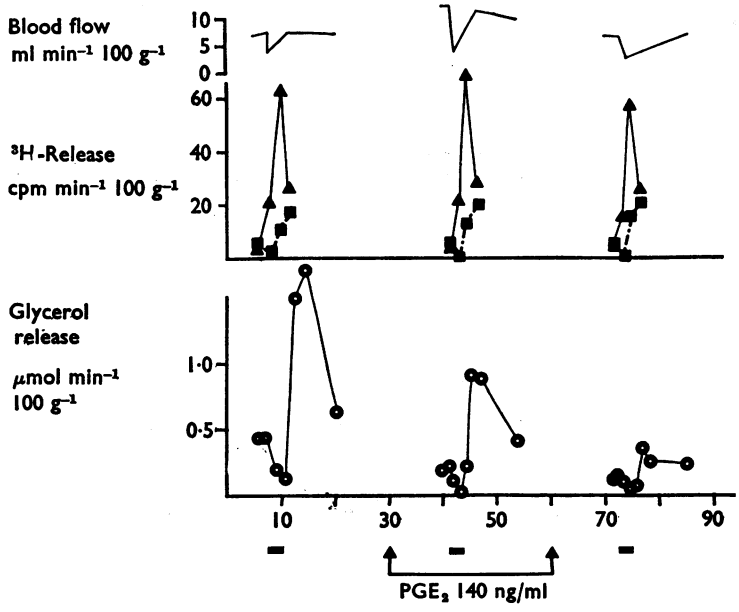

FIG. 1. The effect of prostaglandin E_2 (PGE_2, 140 ng/ml) on blood flow and release of ^{3}H-noradrenaline (▲——▲), ^{3}H-normetanephrine (■- - -■) and glycerol (●——●) before, during and after sympathetic nerve stimulation (4 Hz, 12 V, 2 ms for 2 min) in one experiment.

radioactivity compared with 13% before stimulation (Tables 1 & 2). At the same time there was reduction in the rate of glycerol release. After the cessation of the nerve stimulation there was a marked increase in the overflow of ^{3}H-radioactivity, of which 40% was accounted for by ^{3}H-noradrenaline, and 11% by ^{3}H-normetanephrine (Tables 1 & 2). There was also a marked increase in the rate of glycerol release.

TABLE 1. *Cation exchange column chromatography of radioactivity in close arterial and venous blood of dog subcutaneous adipose tissue previously loaded with ^{3}H-noradrenaline (NA)*

		Venous effluent					Artery	
Drug	Chromatograph product	No. experiments	Pre-stimulation	During stimulation	Post-stimulation 0–2 min	Post-stimulation 3–5 min	Pre-stimulation	No. experiments
0	Acids	6	76·1±1·8	52·0±2·8	48·5±1·8	60·5±3·0	74·0±4·2	3
	NA		13·0±1·5	39·0±3·1	40·1±2·5	25·6±3·7	18·0±5·2	
	NM		10·9±0·4	9·0±0·6	11·4±1·4	13·9±1·7	8·0±1·2	
PGE_2	Acids	3	78·8±0·7	47·0±7·1	45·7±2·0	63·8±0·4		
	NA		11·4±0·7	45·7±6·2	39·6±0·5	19·8±0·6		
	NM		9·8±0·3	7·3±0·3	14·7±2·1	16·4±0·6		
PBA	Acids	3	73·7±2·4	23·3±4·8	27·4±1·8	48·0±2·1		
	NA		16·8±1·6	71·9±4·9	64·7±3·3	39·9±3·8		
	NM		9·5±1·5	4·8±0·5	7·9±1·9	12·1±1·7		
PBA + PGE_2	Acids	3	63·8±0·9	29·2±4·5	31·7±2·6	49·7±4·5		
	NA		24·7±0·9	65·8±4·8	59·9±3·4	39·6±5·9		
	NM		11·5±1·7	5·0±0·3	8·4±1·7	10·7±1·9		

Blood samples withdrawn before, during, and after nerve stimulation. Chromatographic values presented as relative distribution (per cent) of recovered 'acids' (mainly deaminated products), *cf.* Fredholm & Rosell, 1970b, intact noradrenaline (NA) and normetanephrine (NM), and given as means ± S.E.M. PBA=phenoxybenzamine.

TABLE 2. *Canine subcutaneous adipose tissue, loaded with ^{3}H-noradrenaline and autoperfused with blood* in situ. *Effect of nerve stimulation on radioactivity recovered from venous effluent in control experiments after treatment with prostaglandin E_2 (PGE_2) and/or phenoxybenzamine*

	% Change of radioactivity in venous effluent			
	Control	PGE_2	Phenoxybenzamine	Phenoxybenzamine + PGE_2
During stimulation	−17± 8	−5±11	+416±159	+137±40
Poststimulation 0–2 min	+82±18	+125±29	+249± 49	+70±11

Values presented as % change of prestimulation activity and given as means ± S.E.M. n=3–6.

TABLE 3. *The effect of prostaglandin E_2 (PGE_2) on blood flow and glucose uptake in canine subcutaneous adipose tissue*

	Control	PGE_2 (10–15 ng/ml)	% Change	Control	PGE_2 (100–200 ng/ml)	% Change
Blood flow ml min^{-1} 100 g^{-1}	11·3±3·2 (4)	20·0±9·5 (4)	+95±33	5·5±1·0 (8)	12·6± 2·3 (8)	+145±37
Glucose uptake µ mol min^{-1} 100 g^{-1}	8·1±4·2 (3)	14·6±7·5 (3)	+143±60	7·5±2·8 (5)	22·5±10·0 (5)	+160±45

Figures within parentheses number of observations.

Effect of prostaglandin E_2

The infusion of prostaglandin E_2 (10–200 ng/ml) approximately doubled blood flow and glucose uptake (Table 3). In some experiments prostaglandin E_2 decreased the rate of glycerol release from unstimulated adipose tissue (e.g. that illustrated in Fig. 1), but on the average there was no significant inhibition of unstimulated lipolysis. On the other hand, stimulated lipolysis was consistently and dose-dependently inhibited by prostaglandin E_2 (Fig. 2). The inhibition of stimulated lipolysis persisted for a considerable time, although other parameters returned to their control levels (Fig. 1). A similar prolonged inhibition of stimulated lipolysis

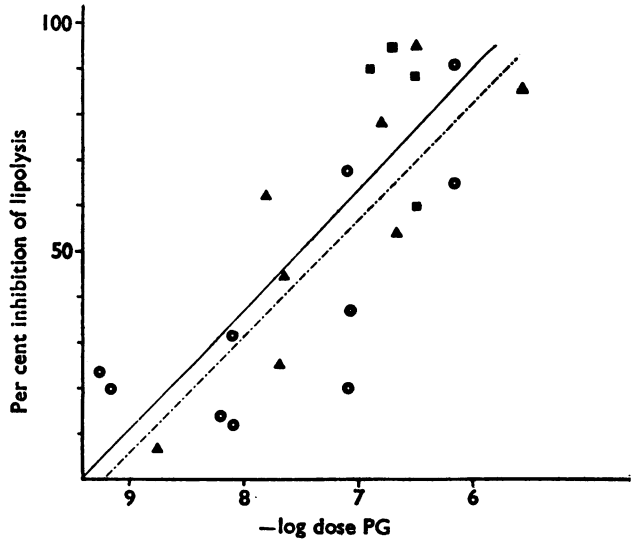

FIG. 2. The relationship between the dose of prostaglandin E_1 (data from Fredholm & Rosell, 1970a) and prostaglandin E_2 and the degree of inhibition of lipolysis induced by sympathetic nerve stimulation. The solid line is the calculated regression of % inhibition on −log dose of prostaglandin E_2 (PGE_2) and the broken line the similar regression for prostaglandin E_1 (PGE_1). ▲—PGE_2 present; ■—PGE_2 plus phenoxybenzamine present; ●—PGE_1 (Fredholm & Rosell, 1970a).

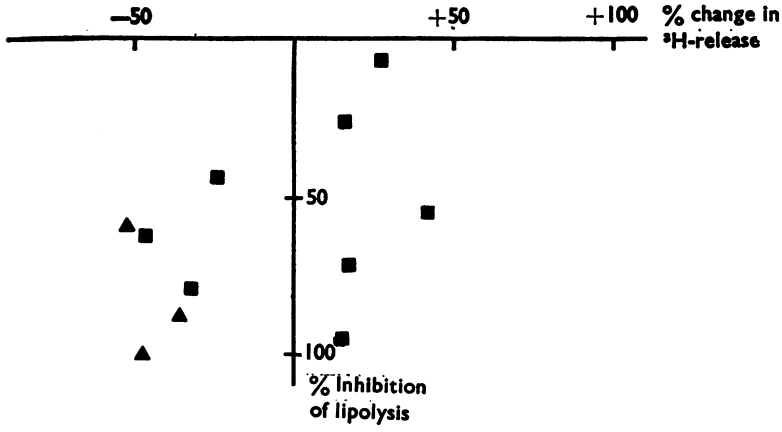

FIG. 3. Effect of prostaglandin E_2 (PGE_2) on lipolysis and release of ^3H in response to nerve stimulation. Values expressed as % change from control. Each point represents the result of one experiment. (■ Without, ▲ with phenoxybenzamine, 1·5–2·0 mg i.a.)

was produced by similar concentrations of prostaglandin E_1 (Fredholm & Rosell, 1970a).

The effect of prostaglandin E_2 on transmitter overflow and vasoconstrictor response to nerve stimulation varied considerably from one experiment to the other. Thus in three cases prostaglandin E_2 caused a clear-cut reduction of induced ^{3}H-noradrenaline overflow while in 5 other cases the transmitter overflow was moderately to markedly increased (Fig. 3). Similarly there was no clear-cut change in the distribution of radioactive overflow (Table 1). In 5 out of 8 experiments the effect of prostaglandin E_2 on induced vasoconstriction varied inversely with that of transmitter outflow.

Effect of phenoxybenzamine and prostaglandin E_2

In order to prevent the vasomotor response to nerve stimulation, phenoxybenzamine was injected i.a. in a dose of 1·5–2·0 mg in three experiments. The vasoconstrictor effect of nerve stimulation was then reduced to approximately 5% of a preceding control observation, while the ^{3}H-noradrenaline overflow response showed a 5-fold increase (Fig. 4). Phenoxybenzamine also caused a marked change in the distribution of radioactivity during and following nerve stimulation (Table 1), and also a change in the time-course so that the largest part of the release was seen during, not after nerve stimulation (Table 2). This change in the release pattern is similar to that seen in constant flow experiments after dihydroergotamine and phentolamine (Fredholm & Rosell, 1970b).

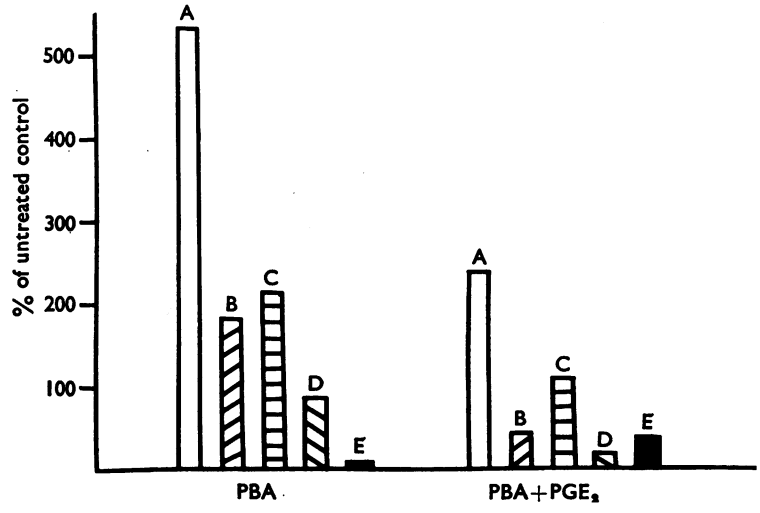

FIG. 4. Cumulative results of three experiments where phenoxybenzamine (1·5–2·0 mg i.a.) was administered. The results are given as % of the effects obtained by nerve stimulation before phenoxybenzamine (PBA). Effect of nerve stimulation on ^{3}H-noradrenaline release (A), ^{3}H-normetanephrine release (B), ^{3}H-total release (C), glycerol release (D) and vasoconstriction (E). All the effects of prostaglandin E_2 (PGE_2) are significant at the 5 per cent level by Student's *t* test for paired variables.

After phenoxybenzamine the evoked glycerol release averaged 90% of the control response (Fig. 4). Since other adrenergic α-adrenoceptor blocking agents, such as dihydroergotamine and phentolamine, markedly potentiate the lipolytic response to nerve stimulation in canine subcutaneous adipose tissue (Fredholm & Rosell,

1968), this finding suggests that phenoxybenzamine, in the dose used, caused an inhibition of lipolysis.

After pretreatment with phenoxybenzamine, prostaglandin E_2 depressed the release of glycerol induced by nerve stimulation to virtually the same extent as seen in the absence of phenoxybenzamine (Figs. 2 and 4). Prostaglandin E_2 also consistently and markedly counteracted the stimulant effect of phenoxybenzamine on the release of total radioactivity and ^{3}H-noradrenaline in response to nerve stimulation, leading to output figures approaching those obtained during preceding control stimulations in the absence of drug treatment (Fig. 4). In spite of its action on transmitter overflow, prostaglandin E_2 antagonized the adrenolytic effect of phenoxybenzamine and partly restored the vasoconstrictor response to nerve stimulation, thus enforcing the inverse correlation between prostaglandin E_2 action on transmitter overflow and vasoconstrictor response to nerve stimulation.

Discussion

There is considerable evidence that stimulation of lipolysis in white adipose tissue is associated with the release of prostaglandins of the E type (Shaw & Ramwell, 1968 ; Christ & Nugteren, 1970 ; Fredholm *et al.*, 1970). Recent evidence suggests that prostaglandin E_2 is the major component of this efflux (Christ & Nugteren, 1970). In the present study it was found that prostaglandin E_2 caused an increased blood flow and glucose uptake as well as a dose-dependent inhibition of lipolysis induced by sympathetic nerve stimulation in canine subcutaneous adipose tissue *in situ*. The effects of prostaglandin E_2 are qualitatively similar to those produced by prostaglandin E_1 in the same preparation (Fredholm & Rosell, 1970a). In particular, prostaglandin E_2 appeared to be at least as potent as prostaglandin E_1 as an inhibitor of stimulated lipolysis.

Before the administration of phenoxybenzamine, prostaglandin E_2 produced no consistent effect on the release of ^{3}H-noradrenaline or on total radioactivity in response to nerve stimulation. After phenoxybenzamine, prostaglandin E_2 caused a significant reduction of transmitter overflow to nerve stimulation, but also a considerable potentiation of the vascular resistance. Admittedly, phenoxybenzamine was administered as a single injection but it seems unlikely that the inhibition it produced had worn off in view of the reported irreversible action of this drug (Triggle, 1971). In other experiments (Fredholm, unpublished) its α-adrenoceptor blocking action was found to last for at least two hours. Since it is known that changes in vascular resistance affect transmitter overflow (Rosell, Kopin & Axelrod, 1963 ; Folkow, Häggendal & Lisander, 1968) it is possible that the reduction in transmitter overflow that was caused by prostaglandin E_2 after phenoxybenzamine was, at least partly, due to potentiation of the vasoconstrictor response. Even in the absence of phenoxybenzamine, prostaglandin E_2 tended to cause oppositely directed changes in vasoconstriction and transmitter overflow during nerve stimulation. For these reasons it is difficult to decide whether prostaglandin E_2 actually caused any reduction in transmitter overflow in canine subcutaneous adipose tissue.

There is much circumstantial evidence to suggest that prostaglandins play a role as modulators of lipolysis in adipose tissue (Bergström *et al.*, 1968 ; Iliano & Cuatrecasas, 1971). The present finding that prostaglandin E_2 even in low concentrations is a potent inhibitor of the lipolysis produced by sympathetic nerve stimulation in adipose tissue *in situ* supports this view. However, as discussed elsewhere

(Fredholm & Rosell, 1970a), it is at present difficult to define the role played by endogenous prostaglandins in the lipolytic response to sympathetic nerve stimulation in subcutaneous adipose tissue of the dog.

In conclusion, prostaglandin E_2 was found to cause a consistent and dose-dependent inhibition of lipolysis and variable changes of transmitter overflow induced by sympathetic nerve stimulation in subcutaneous adipose tissue of the dog. After pretreatment with phenoxybenzamine, prostaglandin E_2 caused an inhibition of lipolysis of equal magnitude and also a clear-cut and consistent reduction of the transmitter overflow response as well as potentiation of the vasoconstrictor response. The evidence suggests that prostaglandin E_2 modifies the lipolytic response to sympathetic nerve stimulation predominantly by a postjunctional action.

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