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Relative activity of prostaglandins E_1, A_1, E_2 and A_2 on lipolysis, platelet aggregation, smooth muscle and the cardiovascular system

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The relative activities of four prostaglandins (PGE_1, PGA_1, PGE_2 and PGA_2) were determined in several biological tests. They were compared as intestinal muscle stimulants on rabbit duodenum and guinea-pig ileum, as inhibitors of adrenaline-induced lipolysis in rat isolated epididymal fat, inhibitors of platelet aggregation in rabbit plasma, as vasodepressor agents in anaesthetized rats and dogs, and on both blood pressure and cardiac output in unanaesthetized dogs. Formation of PGAs by dehydration and introduction of one additional double bond virtually abolished activity in all of these systems except the cardiovascular system. PGE_2 was more active than PGE_1 on isolated rabbit duodenum and as an antilipolytic agent, but less active in the other systems. Only PGE_1 had high potency as an inhibitor of platelet aggregation.

Prostaglandins are a group of acidic lipids, widely distributed in mammalian tissues, which are highly active in many diverse biological tests. Prostaglandin E_1 (PGE_1) is the most thoroughly studied member of the group, and shows outstanding activity as a smooth muscle stimulant, a nasal vasoconstrictor, a vasodepressor agent, and as an inhibitor of gastric secretion, lipolysis and platelet aggregation. Although published comparisons of natural prostaglandins have, in general, revealed only quantitative differences, preliminary studies on a series of synthetic prostaglandin analogues and derivatives of natural prostaglandins indicate that there are also some qualitative differences (Pike, Kupiecki & Weeks, 1967). The pharmacology of the prostaglandins has recently been extensively reviewed (Pickles, 1967; Bergström, Carlson & Weeks, 1968).

The PGA compounds are analogues of the corresponding PGE compounds wherein an additional double bond is introduced at C 10:11 by dehydration (Fig. 1).

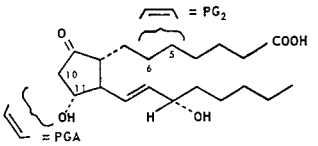

FIG. 1. Structure of prostaglandin E_1 (PGE_1). PG_2s have an additional *cis* double bond at C 5:6. The PGAs are dehydrated derivatives with a double bond at C 10:11 (for nomenclature see Nugteren, van Dorp & others, 1966; Hamberg & Samuelsson, 1967).

The PG_1 and PG_2 compounds differ in that the latter have an additional *cis* double bond at C 5:6. Because of the provocative pharmacological activities of the prostaglandins and possible qualitative differences between some of them, we undertook

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Table 1. *Relative activity of four prostaglandins as smooth muscle stimulants, inhibitors of lipolysis and vasodepressor agents*

Prostaglandin	*In vitro* concns (ng/ml) or *in vivo* doses (μg/kg i.v.)	No. replicate comparisons*	Average response†	Relative activity ($PGE_1=1{\cdot}0$)	95% confidence limits	λ‡
			Rabbit isolated duodenum			
E_1	18		24·1			
	56		34·3			
A_1	1000		21·6	0·010	0·0060–0·016	
	3200		27·3			
		7				0·26
E_2	5·6		20·6	3·1	1·9–5·0	
	18		37·7			
A_2	56		13·0	0·084	0·040–0·14	
	180		21·6			
			Guinea-pig isolated ileum			
E_1	1·8		24·6			
	5·6		48·8			
A_2	320		16·6	0·0020	0·0012–0·0028	
	1000		27·1			
		9				0·22
E_2	1·8		25·3	0·85	0·60–1·19	
	5·6		43·4			
A_2	320		31·2	0·0057	0·0040–0·0080	
	1000		42·6			
			Rat epididymal fat			
E_1	10		72·1			
	100		46·0			
A_1	1000		82·5	0·0037	0·0022–0·0060	
	3200		72·5			
	10000		54·6			
		3				0·18
E_2	10		55·8	2·9	1·7–5·2	
	100		38·8			
A_2	1000		63·6	0·029	0·018–0·049	
	3200		45·3			
	10000		33·4			
			Rat blood pressure			
E_1	1·8		20·0			
	5·6		31·0			
A_1	1·8		13·3	0·30	0·12–0·53	
	5·6		17·7			
		6				0·28
E_2	1·8		2·0	0·13	0·04–0·27	
	5·6		15·7			
A_2	1·8		12·0	0·33	0·14–0·58	
	5·6		20·7			
			Dog blood pressure			
E_1	0·10		14·0			
	0·32		45·3			
A_1	0·018		7·7	2·7	1·5–4·1	
	0·056		24·0			
		6				0·22
E_2	0·18		22·7	0·71	0·46–1·13	
	0·56		45·2			
A_2	0·018		8·0	2·5	1·4–3·9	
	0·056		21·7			

a quantitative comparison in several tests systems of PGE_1, E_2, A_1 and A_2. *In vitro*, they were compared as smooth muscle stimulants (rabbit duodenum and guinea-pig ileum), as antilipolytic agents against adrenaline-stimulated lipolysis in rat epididymal fat and as inhibitors of platelet aggregation in platelet-rich rabbit plasma. *In vivo*, they were compared as depressor agents in anaesthetized rats and dogs, and in unanaesthetized dogs for their effect on both blood pressure and cardiac output.

EXPERIMENTAL

PGE_1 and PGE_2 were prepared by biosynthesis using sheep vesicular glands and the appropriate precursor fatty acid (Daniels & Pike, 1968). PGA_1 and PGA_2 were prepared from the respective PGE compound by heating with glacial acetic acid (Pike & others, 1967).

Methods.† Isotonic contractions of longitudinal segments of rabbit duodenum and guinea-pig ileum were recorded kymographically. Two concentrations of each prostaglandin were selected, separated by 0·5 units log concentration, such that the lower concentration gave small but distinct responses. Relative potencies were calculated from the movement of the writing point (mm).

Antilipolytic activity was evaluated using pooled, chopped epididymal fat of rats, incubated for 1 h with 0·1 μg/ml of adrenaline (Walk, Schultz & Weeks, 1968). Lipolysis was measured by glycerol production. Relative potencies were calculated from glycerol production, but are expressed in Table 1 as % of glycerol formed in presence of adrenaline alone (average μ30·6 mole/g h^{-1}).

Inhibition of platelet aggregation was evaluated using ADP-induced aggregation in platelet-rich rabbit plasma by the revolving-loop method of Silver (1965). The times required for the appearance in succession of visible aggregation, "snowstorm phenomenon" and platelet-head formation were recorded. The prostaglandin concentrations tested were increased progressively (100 μg/ml maximum) until the time for appearance of either visible aggregation or the snowstorm phenomenon differed significantly ($P < 0{\cdot}05$) from control values. Since relative potencies were estimated comparing single threshold concentrations, further statistical evaluation was not possible. However, differences between prostaglandins were so great that more detailed evaluation seemed unnecessary.

The vasodepressor activity in anaesthetized dogs and rats was evaluated using pentobarbitone anaesthetized, pentolinium tartrate pre-treated, vagotomized animals. Femoral (dogs) or carotid (rats) arterial pressures were recorded on a polygraph. Prostaglandins were injected into a femoral vein, using the same experimental design as for isolated intestine experiments. Calculations were based upon mm Hg fall in pressure.

† Details of the several experimental methods used as well as a complete tabulation of experimental results and their statistical interpretations are available on specific request with authors' reprints or order NAPS Document 00185 from ASIS National Auxiliary Publications Service, c/o CCM Information Sciences, Inc., 22 West 34th Street, New York, New York 10001; remitting $1.00 for microfiche or $3.00 for photo copies.

Footnotes to table

* Indicates number of muscles, dogs or rats used. In fat tissue, number of replicate vessels each concentration.

† Isolated muscles; mm pen deflection; fat tissue, glycerol production as % of adrenaline stimulated control; rat and dog blood pressure, mm Hg fall.

‡ Standard deviation/slope, an estimate of the precision of the assay.

Vasodepressor activity was also evaluated in four trained unanaesthetized dogs. Blood pressure was measured from a femoral artery by needle puncture. No more than two doses (one compound) could be tested at a session, since dogs would not remain quiet longer than 15 to 20 min. Every dog received two doses of each prostaglandin separated by 0·5 log interval. A four-point assay calculation could not be used because the doses were not the same in all dogs. Individual dogs varied in sensitivity to the prostaglandins. Furthermore, doses which caused over 30 mm Hg fall in pressure often disturbed the dogs, and their moving or struggling masked effects of the prostaglandin. Consequently, low doses sometimes elicited only equivocal responses. The rank order of potency was therefore estimated graphically by plotting mm Hg fall against log dose for each dog, giving greatest weight to doses which produced approximately equivalent effects.

Effects on cardiac output and blood pressure were evaluated in four trained, unanaesthetized dogs with chronically implanted electromagnetic flow-probes on the ascending arch of the aorta and chronic indwelling cannulas in the abdominal aorta. Only a single dose of each prostaglandin was given to each dog. From the dose and magnitude of the response, the rank order of potency was estimated.

RESULTS

The assay results and relative activities of PGA_1, PGE_1 and PGA_2 relative to PGE_1 as smooth muscle stimulants, inhibitors of lipolysis and vasodepressor agents are summarized in Table 1. All four prostaglandins were qualitatively alike as vasodepressor agents, but the PGAs were very weak as smooth muscle stimulants or inhibitors of lipolysis. Relative activities and other statistical interpretations were derived by treating the data as a parallel-line assay with a randomized block design (Finney, 1964). In all cases there was a clear relation between the effect and the concentration or dose, and the values for λ, ranging between 0·18 and 0·28, are reasonably good for these types of assays.

Table 2 summarizes the effectiveness of the prostaglandins as inhibitors of platelet aggregation. Relative potencies are calculated from the minimally effective concentrations. Only PGE_1 was a potent inhibitor of platelet aggregation.

Table 2. *Relative activity of four prostaglandins as inhibitors of ADP-induced aggregation of rabbit platelets*

Prostaglandin	Concentration μg/ml	Time(s) ± s.e. until appearance of: Visible aggregation	"Snowstorm phenomenon"	Platelet head formation	Relative activity (PGE_1 = 1·0)
Control ..		18·3 ± 1·4	25·3 ± 2·3	32·8 ± 3·5	
PGE_1 ..	0·05	27·7 ± 2·8	>40*	---†	
PGA_1 ..	90	18·0 ± 0·7	>57*	---†	0·0006
PGE_2 ..	10	21·2 ± 1·7	42·8 ± 9·1*	>59*	0·005
PGA_2 ..	100	14·0 ± 1·8	22·2 ± 4·0	25·2 ± 2·3	inactive (<0·0005)

* Difference from control significant at $P < 0·05$.

† Change did not occur.

Figures are based upon the average of replicate values in plasma from 6 rabbits for PGE_1 and PGE_2, 5 rabbits for PGA_2 and 4 rabbits for PGA_1.

As vasodepressor agents on unanaesthetized dogs, the activity of the prostaglandins ranked $PGA_1 = PGA_2$ slightly $> PGE_1 > PGE_2$. In all four dogs PGE_2 was clearly the least active, and in two dogs there was virtually no difference between the PGAs and PGE_1. One of the dogs was uniformly more sensitive to the prostaglandins. In this dog the doses used for PGE_1, PGA_1 and PGA_2 ranged between 0·10 and 0·56 μg/kg, for the other dogs between 0·56 and 5·6 μg/kg. PGE_2 was also more active in this dog than in the other dogs. Unfortunately, there were no comparative data available to determine whether the greater sensitivity in this dog was specifically for prostaglandins or for all vasodepressor agents.

Blood pressure and cardiac output measurements were made after 3·2 μg/kg i.v. of PGE_1, PGA_1 and PGA_2 and after 5·6 μg/kg i.v. of PGE_2. There was considerable individual variation in responses, but for all prostaglandins the peripheral resistance decreased since blood pressure fell and cardiac output increased. The rank order was the same as for dogs in which only blood pressure was measured.

DISCUSSION

The influence of structural changes on biological activities of these prostaglandins may be considered from two aspects: (1) the dehydration and introduction of a double bond at C 10:11 (PGE to PGA); and (2) the presence of an additional *cis* double bond at C 5:6 (PG_1 to PG_2).

Dehydration to PGAs causes the most striking changes in activity. There is virtual loss of contractile activity on intestinal smooth muscle, as well as on inhibition of lipolysis and platelet aggregation. On the other hand vasodepressor activities of the PGAs are relatively unaffected, being only about one-third less than PGE_1 in the rat and nearly three-fold greater than PGE_1 in the dog. The preliminary observations previously reported for PGA_1 are confirmed (see Kloeze, 1967; and Table 1, Bergström & others, 1968).

These results are in agreement with the lack of antilipolytic activity of PGA_1 *in vivo* in the dog (Steinberg & Pittman, 1966). Likewise, the greater *in vivo* vasodepressor activity of PGA_1 seen here agrees with its greater *in vitro* relaxing action on dog isolated arterioles (Strong & Bohr, 1967).

The influence of the additional double bond in the PG_2s is not always the same. PGE_2 was more active than PGE_1 on rabbit duodenum but not on guinea-pig ileum. This observation is in agreement with more limited comparisons previously reported (see Table 1; Bergström & others, 1968). As an antilipolytic agent, PGE_2 was significantly more active than PGE_1, but as a vasodepressor agent, less active. The intestinal muscle stimulant and antilipolytic activities of PGA_2, while very weak relative to PGE_1, were several fold greater than PGA_1. This increase was true even in the guinea-pig ileum, which showed little difference between PGE_1 and PGE_2.

Of the four prostaglandins, only PGE_1 was a potent inhibitor of platelet aggregation. Hampton, Harrison & others (1967) reported that several unrelated vasodilators also inhibited platelet aggregation. Since the PGAs are virtually inactive as inhibitors of platelet aggregation and yet at least equal to PGE_1 as vasodilators, no parallelism between vasodilatation and inhibition of platelet aggregation appears to exist.

The comparison of these four prostaglandins in several systems shows that there is no consistent relation between activities and chemical structure.

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