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Phospholipase inhibition and prostacyclin generation by gastric muscularis and mucosa layers

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Abstract

The effects of drugs which interfere with arachidonate metabolism as well as glucocorticoid-induced anti-phospholipase proteins (APP) have been studied on PGI_2 generation by rat stomach tissue. Indomethacin inhibited PGI_2 generation both *in vitro* and *ex vivo* while dexamethasone was ineffective in both instances. APP inhibited PGI_2 generation *in vitro*. The results are discussed in the light of the possible mode of action of glucocorticoids.

Prostacyclin (PGI_2) is the major cyclo oxygenase metabolite in the rat gastric mucosa [1] and exerts gastroprotective actions [2]. Therefore a correlation between the inhibition of PGI_2 synthesis and the induction of gastric damage has been suggested for the non-steroidal anti-inflammatory drugs [3].

Glucocorticoids inhibit phospholipase A_2 (PLA_2) by inducing in the target cells the synthesis of inhibitory proteins, the lipocortins, [4] and consequently reduce the release of eicosanoids in a number of cells and tissues (for review of this topic, see Ref. 5). However, there is a surprising paucity of information on the effect of glucocorticoids on arachidonic acid (AA) metabolism in the gastro-intestinal tract. Moreover, the relationship between steroid administration and gastic damage is still controversial [6].

The present work was undertaken to investigate the effect of drugs which interfere with AA metabolism on the synthesis of PGI_2 by rat stomach mucosa and by the underlying muscularis layer both *in vitro* and *ex vivo*.

Materials and methods

In vitro experiments

Male Wistar rats (140–160 g), fasted overnight, were killed by cervical dislocation. The stomach was removed, the corpus carefully cleaned from the serosal side and rinsed with ice cold Tris buffer 50 mM pH 8, 4 [7]. Mucosae were stripped off and the underlying muscularis layer was used immediately for PGI_2 generation. The tissue was weighed, chopped with scissors, vortexed for 30 sec, rinsed three times and resuspended in the Tris buffer in Eppendorf tubes. The tubes were then incubated at 37°C in a shaking water bath (160 rev min^{-1}) for 10 min. Drugs or APP were added to the Tris buffer in which the tisues were chopped, rinsed and incubated. At the end of the incubation the tubes were centrifuged (20 sec, 9000 g) and the content of PGI_2 in the supernatant was determined as described below.

Ex vivo experiments

The animals were treated either with indomethacin or dexamethasone as reported in Table 2. The abilities of both gastric mucosa and muscularis layer to synthetize PGI_2 were separately assessed according to the method described by Whittle [3]. Briefly, the tissues were washed, chopped and rinsed 2 times with Tris buffer and vortexed for 30 sec. After centrifugation (20 sec, 9000 g) the supernatant was bioassayed for PGI_2 content. In some experiments on the muscularis layer, tissues were not rinsed but only vortexed.

PGI_2 bioassay

The supernatants from *in vitro* and *ex vivo* experiments were tested for their ability to inhibit aggregation of rabbit platelets (0,5 ml of citrated platelet-rich plasma) induced by adenosine diphosphate (10–20 μM) using an Elvi dual-channel aggregometer. This inhibition was assayed against authentic PGI_2 (as sodium salt) dissolved in 1 M Tris buffer, pH 9,5 at 4°C and freshly diluted with 50 mM Tris buffer, pH 8,4 at 4°C.

Materials

Dexamethasone sodium phosphate (Decadron) was obtained from Merck, Sharp & Dohme. Adenosine diphosphate, arachidonic acid, indomethacin, bovine serum albumin, mepacrine (quinacrine), tranylcypromine (trans-2-phenylcyclopropylamine), prostacyclin were purchased from Sigma Chemicals, U.S.A.

The anti-phospholipase proteins were collected, and purified as previously described in detail by Blackwell et al. [8] and Parente et al. [9]. The molecular weight of APP I, II and III were respectively 200K, 40K, 15K. The inhibition of PLA_2 by the APP was assessed *in vitro* according to the method of Blackwell et al. [8]. The inhibition observed was: APP I = 65%; APP II = 50%; APP III = 70%.

Results

Table 1 shows the effect of different drugs on the *in vitro* synthesis of PGI_2 by rat stomach muscularis tissue. Indomethacin (cyclo-oxygenase inhibitor), tranylcypromine (PGI_2 synthetase inhibitor) and mepacrine (PLA_2 inhibitor) vir-

tually abolished the PGI_2 generation while dexamethasone and BSA were ineffective. Arachidonic acid (1 μg/ml, n = 3) was able to reverse the inhibitory effect of mepacrine while it was without effect on the inhibition exerted by indomethacin (data not shown). APP I-II-III exhibited a significant inhibitory effect (50–80%) on PGI_2 generation *in vitro*.

Table 2 shows the effect of *in vivo* administration of indomethacin and dexamethasone on the *ex vivo* synthesis of PGI_2 by rat gastric mucosa or muscularis tissue. Indomethacin was able to greatly reduce PGI_2 generation by both tissues, whereas dexamethasone was ineffective in all dose regimens used. The steroid (1 mg Kg−1, 3h pretreatment) was also ineffective when PGI_2 content was assessed from tissues which were vortexed without preliminary rinsing (data not shown). No signs of gastric damage were detected in any of the animals receiving the steroid.

Table 1
Effect of some cyclo-oxygenase- prostacyclin synthetase- and phospholipase- inhibitors, albumin and dexamethasone on *in vitro* PGI_2 generation by slices of muscularis layers of rat stomach corpus. The control release (10 min) was 7.43 ± 0.11 (n = 20) ng mg^{-1} wet weight. Results are expressed as % inhibition of the control release; mean ± s.e. mean of (n) values. Φ = P < 0.01 (calculated on means of values expressed in ng mg^{-1}).

	μg ml^{-1}	% inhibition	(n)
indomethacin	1	100 ± Φ	3
tranylcypromine	60	71 ± 1.6 Φ	4
mepacrine	60	74 ± 2.7 Φ	5
APP I	60	64 ± 2.6 Φ	3
APP II	60	49 ± 1.6 Φ	3
APP III	60	79 ± 3.2 Φ	3
albumin	60	0	4
dexamethasone	60	0	4

Discussion

The results of the *in vitro* experiments show that the pharmacological inhibition of the enzymes involved in generation of AA metabolites leads to a reduction of PGI_2 synthesis by rat gastric tissues *in vitro*. The inhibitory effect of indomethacin confirms previous data [10]. It is of some interest that PLA_2 inhibitors (mepacrine and APP) which reduce AA availability also decrease PGI_2 formation. Peritoneal lavage fluids from steroid-treated rats contain substantial amounts of APP which can be fractionated by chromatography [8, 9]. All the different anti-PLA_2 fractions (APP I-III) were able to inhibit PGI_2 generation *in vitro*. Dexamethasone had no effect *in vitro*, confirming its ineffectiveness on smashed tissues [10]. The lack of inhibitory effect of dexamethasone treatment *in vivo* agrees with previously reported results [11]. This finding is difficult to explain since *in vivo* the steroid is able to induce APP which in our *in vitro* experiments inhibited PGI_2 generation. The wash-out during the tissue rinsing of the APP possibly induced by the steroid cannot explain the failure of dexamethasone treatment since the steroid was also ineffective in tissues without previous rinsing. A possible explanation for the ineffectiveness of dexamethasone in reducing PGI_2 generation *ex vivo* could be the absence in normal rat stomach

Table 2
Effect of indomethacin (orally) and dexamethasone (subcutaneously) on prostacyclin generation by rat gastric mucosa (a) and by underlying muscularis layer (b). The control PGI_2 generation was: (a) 0.15 ± 0.01 ng mg^{-1} (n = 15); (b) 0.97 ± 0.04 ng mg^{-1} (n = 20). The changes in PGI_2 generation in treated animals were given as % change compared to control generation. The results are expressed as means ± s.e.m. of (n) values. Φ = P < 0.01 (calculated on means of values expressed in ng mg^{-1}).

	time of pretreatment		% inhibition	
	mg kg^{-1}	(h)	(a)	(b)
indomethacin	3	3	85 ± 1.9(7) Φ	94 ± 0.3(6) Φ
dexamethasone	1	1	5 ± 4.9(8)	0 ± 1.2(3)
,,	1	3	7 ± 4.2(10)	1 ± 1.1(10)
,,	1	6	3 ± 4.6(10)	3 ± 1.4(4)
,,	1	8	5 ± 4.5(8)	4 ± 3.4(5)
,,	1	12	+2 ± 4.9(10)	5 ± 4.0(6)
,,	1	24	10 ± 5.3(10)	6 ± 2.1(7)
,,	1 × 4(′)	3	8 ± 7.4(5)	0 ± 1(5)

(′) daily, from 4 day before

tissues of target cells capable of responding to steroid stimulation by synthetizing APP. Indeed it has been shown that glucocorticoids do not affect the basal rate of prostanoid synthesis *in vivo* [12]. Thus it appears conceivable that glucocorticoids can act *in vivo* only by preventing the PLA_2 activation which occurs, for instance, in inflammatory reactions.

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