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Nitrates do not affect prostacyclin formation by rat arteries: this is unrelated to increased vascular prostacyclin formation with age

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The question of whether vasodilator nitrates act by releasing prostacyclin is controversial. Since the ability of blood vessels to form prostacyclin changes with age, we have investigated whether this may explain the discrepancies in the literature. It does not, since isosorbide dinitrate or glyceryl trinitrate incubated with rat aorta or vena cava from male Wistar rats had little or no effect on the release of prostacyclin, measured as 6-keto-$PGF_{1\alpha}$. We confirm that the aorta produces substantially more prostacyclin than the vena cava. The arterial production of prostacyclin was greater in rats weighing 350–400 g than in those weighing 116–152 g, but the production by the veins was similar in both groups.

The effect of nitrates on the vascular production of prostacyclin (PGI_2) is controversial. Studies in-vitro supporting a stimulation of PGI_2 formation by glyceryl trinitrate (GTN) or isosorbide dinitrate (ISDN) are those by Anderson et al (1980), Levin et al (1981), Schror et al (1981) and Nakabayashi et al (1985), although in the latter case isosorbide mononitrate had no effect. However, contrary evidence was obtained by Forster (1980), Neichi et al (1980) and Mayeux et al (1985), and indomethacin did not change the relaxation induced by GTN in rabbit blood vessels (Bennett et al 1983), or by isosorbide mononitrate in dog veins (Bartsch et al 1983).

In man, Forster (1980) found no increase with GTN in circulating prostaglandins, and Fitzgerald et al (1984) found no significant alteration in the urinary excretion of the PGI_2 metabolite 2,3-dinor-6-keto-$PGF_{1\alpha}$ with GTN or ISDN. Indomethacin did not alter the response to nitrates in patients (ISDN, Simonetti et al 1983; GTN, Thadani & Kellerman 1983). In dogs, Nugent et al (1982) found that GTN did not increase 6-keto-$PGF_{1\alpha}$ generation in the coronary sinus blood, and indomethacin did not greatly alter the vasodilatation to nitrates (Morcillo 1980; Morcillo et al 1980).

However, vascular responsiveness and prostacyclin formation change with age. Most studies report increased prostacyclin production as rats get older (e.g. Pace-Asciak & Carrara 1979; Panganamala et al 1981; Adolfs & Elliott 1982; Deckmyn et al 1983; Horvath & Benko 1984). The present experiments have investigated whether age differences explain the discrepancies in the literature about the effect of nitrates on prostacyclin formation by rat isolated arteries and veins.

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Methods

Male Wistar rats of 2 weight ranges were used, 350–400 g (old rats, about 10–15 weeks) and 116–152 g (young rats, about 4–5 weeks). They were fed CRM diet and allowed free access to water. After stunning and exsanguination, the thorax and abdomen were opened and the aorta and vena cava dissected out. The arteries and veins, studied separately, were cut into small rings and washed in Krebs solution. Each experiment used pooled tissues from 8 rats. Aliquots of 20–30 mg tissue were incubated for 30 min in 5 mL Krebs solution containing various concentrations of either isosorbide dinitrate (ISDN) or glyceryl trinitrate (GTN, Tridil). The supernatant was then removed, acidified to approximately pH 3 with formic acid and extracted into chloroform (Unger et al 1971), giving a recovery of 84 ± 2·3% (mean ± s.e.) for 6-keto-$PGF_{1\alpha}$. After evaporating the chloroform under vacuum at 37 °C, the residue was reconstituted with tricene buffer. The samples were measured in duplicate for 6-keto-$PGF_{1\alpha}$ by radioimmunoassay with appropriate procedure blanks, using an antibody to 6-keto-$PGF_{1\alpha}$ (Wellcome Research Laboratories) whose per cent cross-reactivities were: 6-keto-$PGF_{1\alpha}$ 100; $PGF_{2\alpha}$ 3·0; PGE_2 0·1; TXB_2 0·02. The drugs did not interfere with the assay.

In each experiment the tissues were processed and assayed at the same time, and the results are uncorrected for the mean loss of 16 ± 2·3%. The measurements are given to 3 significant figures, and presented as medians with semiquartile ranges in parentheses, or as ranges or actual values for the vena cava from young rats since it was sometimes impossible to obtain tissue and the numbers are small. The intra- and inter-assay coefficients were, respectively, 10 and 21%. Statistical analysis was by the Wilcoxon matched-pairs signed-ranks test or the Mann-Whitney U-test as appropriate (both 2-tailed).

Results

As shown in Tables 1 and 2, aortic tissue from male Wistar rats produced substantially more PGI_2 than the vena cava, measured as 6-keto-$PGF_{1\alpha}$. Older arteries produced more PGI_2 than the younger arteries. The difficulty in obtaining vena cava from the young rats allowed only a few experiments on this tissue. However, since the studies on the veins of young rats were done separately with ISDN and GTN, we can combine the control values to obtain an estimate of PGI_2 production

Table 1. Isosorbide dinitrate (ISDN) incubated with rat aorta or vena cava for 30 min had little or no effect on the amount of 6-keto-$PGF_{1\alpha}$ extracted from incubates (all P values >0·1). The values are medians, with semiquartile ranges in parentheses, except for the young vena cava which are shown as ranges or individual values. However, the control old arteries produced more 6-keto-$PGF_{1\alpha}$ than the old veins ($P < 0·0001$), the young arteries ($P < 0·0001$), and the young veins ($P < 0·002$). Young arteries produced more 6-keto-$PGF_{1\alpha}$ than young veins ($P < 0·002$). The control tissues in these experiments were the same as with glyceryl trinitrate (Table 2) except for the young veins which were studied separately.

	6-keto-$PGF_{1\alpha}$ (ng g^{-1} wet tissue)			
ISDN ng mL^{-1}	Old aorta n = 7	Old vena cava n = 7	Young aorta n = 7	Young vena cava n = 1–4
0	1810 (1790–2030)	191 (150–207)	743 (597–772)	97–203
1	2370 (1990–2880)	179 (161–217)	750 (578–921)	121–241
10	1920 (1760–2400)	290 (193–321)	615 (547–676)	205
100	1910 (1630–2670)	155 (145–214)	675 (643–695)	255
1000	2040 (1510–2670)	175 (171–213)	708 (616–731)	158–373

Table 2. Glyceryl trinitrate (GTN) incubated with rat aorta or vena cava for 30 min had little or no effect on the amount of 6-keto-$PGF_{1\alpha}$ extracted. The values are medians, with semiquartile ranges in parentheses, except for the young vena cava where they are ranges or individual values. All the P values were >0·1 compared to 0 GTN , except for GTN 100 ng mL^{-1} in the young arteries where $P < 0·1$. * n = 6.

	6-keto-$PGF_{1\alpha}$ (ng g^{-1} wet tissue)			
GTN ng mL^{-1}	Old aorta n = 7	Old vena cava n = 7	Young aorta n = 6	Young vena cava n = 0–3
0	1810 (1790–2030)	191 (150–207)	744 (682–770)	93–233
1	2400 (1990–2710)	189 (160–210)	718 (700–785)	122, 246
10	2380 (1280–3050)	177 (133–197)	751 (635–914)	290
100	1810 (1580–2480)	188 (151–240)	660 (606–714)	–
1000	2320 (1610–2460)	123 (113–208)*	606 (526–652)	212–244

from seven different groups of tissues. The median and semiquartile range for the veins from young rats were 189 (97–203) ng g^{-1} 6-keto-$PGF_{1\alpha}$, which was very similar to the 191 (150–207) ng g^{-1} from the old veins ($P > 0·5$). Thus, although the arterial production of PGI_2 g^{-1} tissue increased with age, production from the veins seems to remain constant.

Regardless of age, ISDN and GTN had little or no effect on the amount of PGI_2 released from rat aorta or vena cava.

Discussion

Our experiments with a wide range of concentrations, including those of therapeutic relevance, argue against PGI_2 release in the therapeutic effects of ISDN or GTN. This conclusion agrees with that of Forster (1980), Morcillo (1980), Morcillo et al (1980), Neichi et al (1980), Nugent et al (1982), Mayeux et al (1985), Bennett et al (1983) and Bartsch et al (1983) in laboratory animals, and with Forster (1980), Fitzgerald et al (1984), Simonetti et al (1983) and Thadani & Kellerman (1983) in man. However, as pointed out in the Introduction, others disagree. Our results indicate that the age of the animal is unlikely to account for the different results, at least in rats. We therefore still do not understand the reason for the discrepancies between reported findings, but possible explanations include differences in methodology, species, strains of animals, and seasonal variations.

Our results confirm that incubated segments of aorta produce substantially more PGI_2 than the vena cava, and that aorta from older rats produces more PGI_2 than that from younger rats. However, the production of PGI_2 by the vena cava was similar in both age groups.

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Surface area and crystallinity of Form A of chloramphenicol palmitic and stearic esters: which one is the limiting factor in the enzymatic hydrolysis?

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Chloramphenicol stearic and palmitic esters in the polymorphic Form A, when ground for 85 h showed an in-vitro enzymatic hydrolysis rate constant (K_{hydr}), the value of which was the same as that of a commercial Form B. The increase in the rate of the enzymatic hydrolysis was not related to the specific surface area as shown by the fact that the micronized Form A, having a higher specific surface area value than ground Form A, showed the same K_{hydr} as the unground Form A. The K_{hydr} value of the ground Form A could be the result of an increase in the crystalline disorder brought about by the grinding process.

The therapeutic activity of chloramphenicol (CAP) palmitic (Glazko et al 1952) and stearic (Pauletta 1952) esters is due to their in-vivo enzymatic hydrolysis to free chloramphenicol by pancreatic lipase.

Glazko et al (1958) reported that the in-vitro enzymatic hydrolysis rate of chloramphenicol palmitate depended on the particle surface area. Almirante et al (1960) stated that blood levels reached with the CAP stearic ester were dependent on the polymorphic form used: either a mostly amorphous form that is hydrolysed by enzymes in the gastrointestinal tract or a crystalline form that does not yield useful blood levels of CAP.

Tamura & Kuwano (1961) found two crystalline forms and one amorphous form of CAP palmitic ester; Aguiar et al (1967) found that suspensions containing only Form B gave higher blood levels than those with Form A; those authors also specified that the increase of particle size from 5 to 25 μm had a limited influence on the bioavailability of the drug.

Kelbaek & Ulrich (1969) found that the hydrolysis rate decreased with increasing particle size.

There are different opinions about the mechanism of enzymatic hydrolysis of the CAP esters, but the existence of a biologically active Form B and of a biologically inactive Form A has been proved (Almirante et al 1960; Aguiar et al 1967). The bioactivity differences between the CAP ester polymorphs have been explained by the relatively large free energy difference (Andersgaard et al 1974) and by the different surface properties of the crystals (Kaliszan 1986).

The present work aimed for more information about the factors involved in the enzymatic hydrolysis of the biologically inactive Form A of CAP palmitic and stearic esters.

Materials and methods

As chloramphenicol palmitate (CAP-P) (F.U. IX) and stearate (CAP-S) (Codex Erba) (Carlo Erba), named 'non polimorfo A' by the manufacturer, correspond to the biologically active Form B of both esters, they were used in the present work without purification as commercial CAP-P and CAP-S samples. To prepare the polymorph A, these products were used after repeated crystallizations from toluene–n-hexane and from methanol–water according to Miyamoto et al (1973).

Pancreatin, FIP-lipase 7·5 u mg^{-1} (Merck), was used as received from the manufacturer. All solvents were of analytical grade (Carlo Erba).

Preparation of polymorph A

Unground Form A (A). Form A of CAP-P and CAP-S was obtained according to Borka (1970) by crystallization from chloroform of the commercial purified CAP-P and CAP-S.

Micronized Form A (A_m). The Form A obtained from CAP-S was micronized using a JM-80 (Fryma).

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