INHIBITORY EFFECTS OF ASPIRIN AND INDOMETHACIN ON THE BIOSYNTHESIS OF PGE_2 AND $PGF_{2\alpha}$

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SUMMARY

A gas-liquid chromatography system has been used to study the effects of indomethacin and aspirin on the biosynthesis of PGE_2 and $PGF_{2\alpha}$ by the prostaglandin synthetase system of bovine seminal vesicle. Both compounds were found to inhibit the production of PGE_2 and $PGF_{2\alpha}$. However, based on statistical analyses, the inhibitory effect of indomethacin was found to be non-selective while aspirin produced statistically significant preferential inhibition of PGE_2 over $PGF_{2\alpha}$.

INTRODUCTION

Several groups of investigators have demonstrated inhibition of prostaglandin formation from arachidonic acid by non-steroidal antiinflammatory agents. These studies have employed a variety of tissues including bovine[1,2,3] and ram seminal vesicles[4], dog spleen[3,5], guinea pig lung[6], human skin[7], and rabbit brain[3,8]. Also, a variety of methods for the measurement of prostaglandin produced have been employed, some of which measure only PGE_2 formation, while others estimate PG-like activity.

Recently, quantitative techniques have been employed to simultaneously estimate the amount of prostaglandin formed from arachidonic acid[3]. Flower et al.[3] suggested that the simultaneous study of the products of the action of prostaglandin synthetase could have important physiological implications. Recognizing the differential effects of PGE_2 and $PGF_{2\alpha}$ on various tissues and organ systems, selective inhibition of the synthesis of a specific prostaglandin could conceivably alter the balance in a particular tissue and such a result could be of potential therapeutic interest. To observe selective inhibition

demands accurate analytical test procedures. In the present study a gas-liquid chromatography system was developed to measure the amounts of PGE_2 and $PGF_{2\alpha}$ synthesized from arachidonic acid by bovine seminal vesicle microsomes (BSVM).

The inhibitory effects of aspirin and indomethacin on the production of PGE_2 and $PGF_{2\alpha}$ have been reported previously in studies employing bioassay systems[6], and radiochemical and spectrophotometric analytical methods[3]. The results of the current study suggest that indomethacin inhibits the synthesis of PGE_2 and $PGF_{2\alpha}$ to the same extent, while aspirin inhibits PGE_2 to a greater extent than $PGF_{2\alpha}$.

MATERIALS AND METHODS

1) Biological

A. BSVM were prepared as described by Takeguchi et al.[9], but were stored as frozen suspensions instead of being lyophilized.

B. The biological reaction conditions were also adapted from Takeguchi et al.[9]. The concentration of components are shown below:

Buffer	0.05 M Tris pH 8.3
Arachidonic Acid	0.33 mM
L-epinephrine Bitartrate	2.0 mM
BSVM	4-6 mg/ml
Cutscum	0.04%
Reaction Volume	10 ml
Reaction Time	6 min
Reaction Temperature	37.5° C

In the initial series of experiments the reaction was permitted to continue for different times in order to construct a time curve for the production of PGE_2 and $PGF_{2\alpha}$. The reaction was stopped by acidification with 25% formic acid to a pH of 3.0-3.5 and immersion in an ice bath. In the studies with aspirin and indomethacin the reaction time was uniformly six minutes. The inhibitory effects of these two compounds were studied under two different sets of conditions. First, the enzyme preparation and inhibitor were preincubated at 25°C for five minutes before substrate was

added. Second, the inhibitor was not preincubated with enzyme, but was added directly to the reaction mixture containing both enzyme and substrate. Duplicate samples were run in all experiments. All data presented are for preincubated samples.

2) Analytical

A. The acidified reaction mixture was extracted with ether and was backwashed with distilled H_2O. The ether layer was reextracted with pH 8.0 phosphate buffer. The buffer was then adidified with 25% formic acid to pH 3-3.5 and extracted with ether. The ether was backwashed with distilled H_2O. The GLC internal standard cholanic acid was added at this point and the solution was evaporated to dryness. Diazomethane in ether was added to the residue and allowed to react for 15 minutes. Excess reagent and ether were removed under a stream of N_2 at R.T. The residue was then treated with 2% methoxamine in pyridine at 60°C for ½ hour. The pyridine solvent was removed under a N_2 stream, and bis (trimethylsilyl)trifluoroacetamide and 1% trimethylchlorosilane) (BSTFA and TMCS) (Pierce Chemical Co.) in pyridine was added and reacted for one hour at 60°C. The resulting solution was used for gas chromatographic analysis.

B. The derivatized prostaglandins were analyzed by gas chromatography using an internal standard method. Standard curves were prepared with authentic $PGF_{2\alpha}$ and PGE_2 containing internal standard.

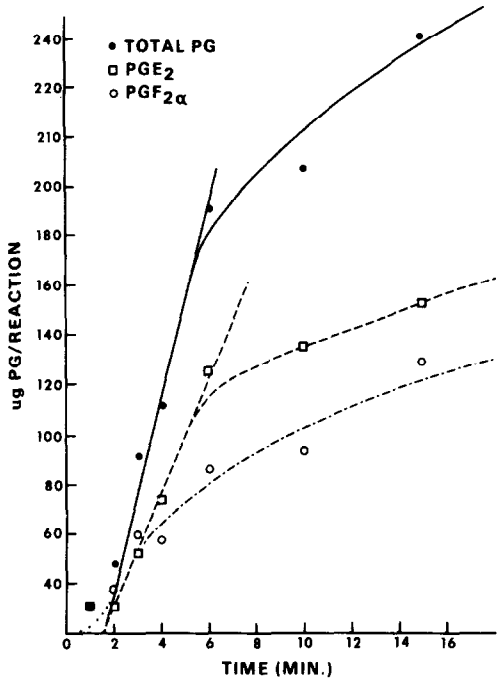
G.C. Conditions

Instrument	Perkin Elmer 900
Column	3% OV 225 100/120 Gas Chrom Q glass 6 ft x 2 mm
Column Temperature	210°C
Flow	20 cc/min He
Detector	F.I.D.
Injector Port	225° (glass)

C. The PGE_2 and $PGF_{2\alpha}$ were obtained from the Hormel Institute or Ono Chemicals (Japan). All other chemicals were reagent grade or the

FIGURE I.

FORMATION OF PROSTAGLANDIN WITH TIME



highest purity commercially obtainable, and were used without further purification.

RESULT AND DISCUSSION

Figure I shows a typical curve for the formation of PGE_2 and $PGF_{2\alpha}$ as a function of time. The percentage conversion of substrate at six minutes is usually 15-20%. Under the reaction conditions described above, the PGE_2:$PGF_{2\alpha}$ ratios typically are 60:40 but vary from 50:50 to 70:30 for any given BSVM preparation.

The data in Figure II are from studies in which the inhibitor was pre-incubated with BSVM. Under these conditions aspirin and indomethacin inhibited the synthesis of both PGE_2 and $PGF_{2\alpha}$. In studies in which the inhibitor was added directly to the reaction mixture containing enzyme and substrate, the inhibitory effects of indomethacin and aspirin were not apparent. This observation is consistent with the work of Flower et al.[3] and Smith and Lands[10] who have noted that incubation conditions are critical in studies of this type.

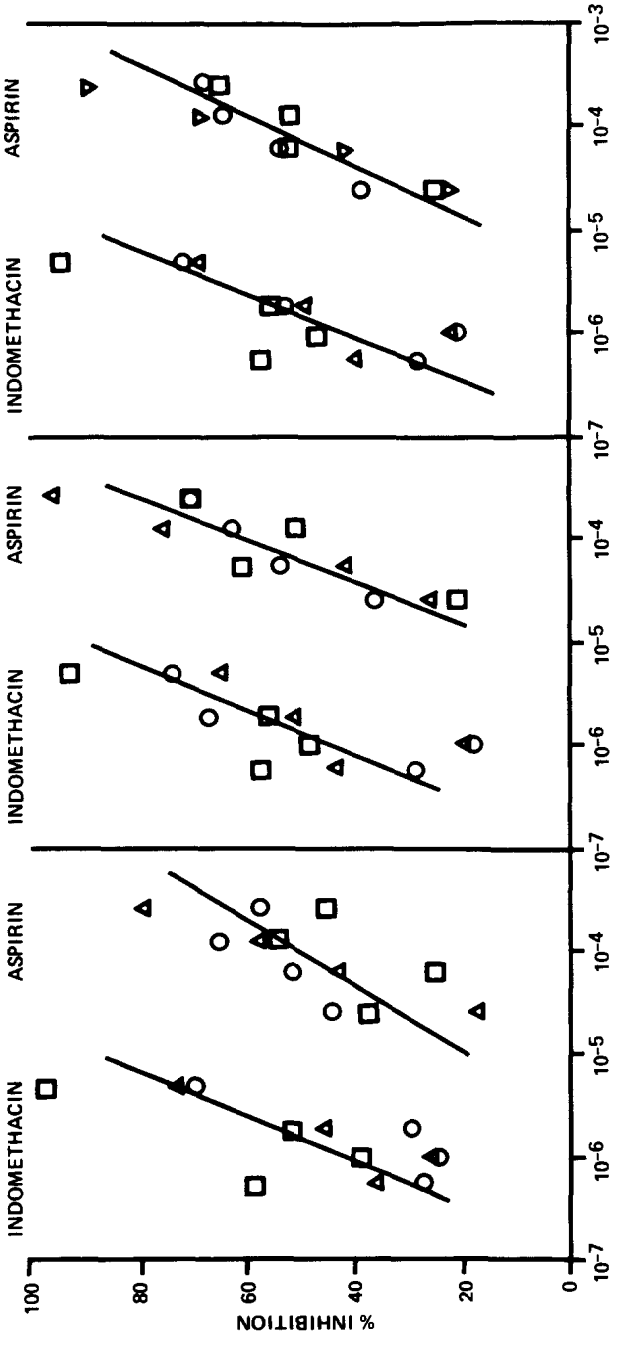


FIGURE II. INHIBITION OF PGE_2, $PGF_{2\alpha}$ AND TOTAL PG SYNTHESIS BY ASPIRIN AND INDOMETHACIN

The ▲, ○ and □ show the experimental points from three different experiments. Each point is an average of duplicates. The percent inhibition of total prostaglandin is calculated from the sum of $PGF_{2\alpha}$ and PGE_2 concentrations in reaction controls and reactions with inhibitor.

	ID_{50} (μM)		Activity Ratio
	Indomethacin	Aspirin	Indomethacin : Aspirin
$PGF_{2\alpha}$	1.7	100	59 : 1
PGE_2	1.3	55	42 : 1
TOTAL	1.4	60	43 : 1
$PGF_{2\alpha}/PGE_2$	1.3	1.8	

TABLE I

Published ID_{50} Values For Aspirin And Indomethacin

Reference	Tissue*	Method of Analysis	ID_{50} (uM)		Activity Ratio**
			Indomethacin	Aspirin	
This work	BSVM	GLC	1.4	60	43:1
(1)	BSVM	Adrenochrome	2.0	820	410:1
(2)	BSVM	Radiochemical	7.0	15000	2143:1
(10)	RSVM	Oxygen electrode	9.0	9000	1000:1

*BSVM = Bovine Seminal Vesicle Microsomes, RSVM = Ram Seminal Vesicle Microsomes
**Activity Ratio = Indomethacin:Aspirin

When the $PGF_{2\alpha}$:PGE_2 ratios for indomethacin and aspirin were analyzed statistically by the method of parallel line bioassay[11], the data obtained indicate that indomethacin produces a non-selective effect while aspirin produces a greater reduction in PGE_2 than in $PGF_{2\alpha}$. The respective ratios and 95% confidence limits for indomethacin and aspirin were found to be 1.3 (0.8-2.3) and 1.8 (1.1-3.2). Selective inhibition of prostaglandins synthetase activity has been reported by others. Wlodawer et al.[12] reported preferential inhibition of PGE_1 as opposed to $PGF_{1\alpha}$ with a prostaglandin-like intermediate. More recently Flower et al.[3] reported that benzydamine was 4.5 times as effective in inhibiting the formation of $PGF_{2\alpha}$ as it was in inhibiting the production of PGE_2. These authors also found that phenylbutazone reduced $PGF_{2\alpha}$ and PGE_2 to a greater extent than two other products of the biosynthetic process, namely, PGD_2 and malondialdehyde.

The potency ratio between indomethacin and aspirin in the current study was found to be 43:1 (using the ID_{50} for total prostaglandin synthesis). In Table I this value is compared to the ratios reported by other workers.[1,2,10]

Reviewing these data (see Table I) it is apparent that, despite the different analytical systems employed, there is reasonably good agreement between ID_{50} values reported for indomethacin. The ID_{50} values for aspirin, however, vary considerably. These differences may be due in large part to the different analytical methods employed, which measure different steps in the reaction sequence. The relatively consistent activity of indomethacin observed by each of these techniques suggests that it inhibits at an early step in the biosynthetic pathway, while the very wide variation in the potency of aspirin indicates that it may inhibit at more than one step. This difference in the site of activity proposed for indomethacin and aspirin is consistent with our earlier observation of a difference in the degree of inhibitory selectivity.

The data of Tomlinson et al.[2] indicate that cofactor may also influence ID_{50} ratios. Here again the ID_{50} of indomethacin is in agreement with other values in Table I, but the ID_{50} of aspirin is much larger. This indicates that cofactor may also influence the activity of an inhibitor.

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