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STUDIES ON THE MODE OF ACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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ABSTRACT

During the past decade the mode of action of anti- inflammatory drugs has mainly been related to the inhibition of prostaglandin (PGS) synthesis (Vane 1971). In a presentation by prune (1982) it has been shown that PGS are not the only most important mediators of inflammation, and the inhibition of prostaglandin synthesis alone does not explain all common effects and mode of action of NSAFDS. Hence alternative concepts are presented in this paper, which may be adopted as the mode of action of NSAIDs.

Some inflammatory conditions of cardiovascular system may be responsive to some NSAIDs like Aspirin Phenylbutazone, Indomelhacin, Ibuprofen, Flurbiprofen. Prostaglandins (PGS) produce inflammatory diseases of heart and vessels but PGS alone may not be the sole cause of the disease but may produce inflammation in con-junction with other suspected mediators of inflammation like SHT, Catecholamines and histamine (vasoactive substances). Inhibition of PGS alone is not sufficient enough to supress inflammation completely. A new generation of NSAIDs can be explored which may be capable of inhibiting perhaps all mediators of inflammation, for which a new approach or the mode of action of the present should be explored. In this presentation effects of above said NSAIDs are evaluated on vasoactive substances (other mediators of inflammation) in order to determine the mode of action of these drugs with a new approach.

Introduction

There are certain cardiovascular ailments of inflammatory nature like, post myocardial infarction pericarditis (P.M.I.P), Phlebitis valvitis, myocarditis thrombo-phelebitis and cerebrovascular diseases. Such disorders may be responsive to Non-steroidal anti- inflammatory drugs like Aspirin, Indomelhacin Phenylbutazone Ibuprofen and Flurbiprofen.

The discovery by Vane in 1971 has shown that NSAIDs, inhibit cyclooxygenasc catalyzed biosynthesis of prostaglandin a mediator of inflammation. Recently it is postulated and presented that there are certain other mediators of inflammation such as SHT, catecholamines and histamine may take part in producing inflammation (Willoughby & Sedgwick 1982).

These vasoactive substances also affect the functioning of the heart and regulate the tone of the vessels. These vasoactive substances are active in inflammatory process as mediators of inflammation (Willoughby & Sedgwick 1982, Hart and Huskisson 1985). Most of the diseases of cardio-vascular system are mediated by vasoaclive substances such as myocarditis of pheochromocytoma (Kline 1961, Vanvliet, Burcheli and Tilus 19M).

Adrenaline and 5HT promote platelet aggregation. Adrenaline stimulates the release of thromboxanc A2 which causes platelet aggregation and acts as a potent-vasoconsrictor (Braunwald 1978). Discharge of 5HT from platelets contribute to pathophysiology of pulmonary embolism (Spodick 1972). Drugs inhibiting 5HT may be useful in controlling hypertension, inflammation and inhibition of platelet aggregation. Depletion of 5HT may causes suppression in inflammatory vascular response. 5HT has been found to stimulate prostacyclin (PGI2) which is basically a vasodilator carrying features of inflammation (Coughlin, Moskowitz, Antoniades and Levine 1981).

Histamine is active in early phase of inflammation and its high concentrations may cause arrythmias and increased capillary permeability exhibiting typical features of inflammation, while catecholamines5HT and histamine have also been hypothesized to be involved in prinzmetal angina (Goodman & Gilman 1985).

Mode of action of most of NSAIDs even of Aspirin is still uncertain, similarly mechamisms involved in different arthopathies are still not exactly known. It may be assumed on the basis of above mentioned statements and facts that NSAIDs which are believed to act by inhibition of PGS (Vane 1971), might be acting on all mediators of inflammation.

These findings possibly would lead to a different approach for studying the mode of action of these drugs. Furthermore this will facilitate the studies on pathophysiologies mediated by these vasoactives agents. This presentation offers a possible different mode of action of few well known NSAIDs.

Materials and Methods

Few well known NSAIDs are evaluated on healthy rabbits weighing 1.5-2.0 Kg. Selected rabbits are conditioned in laboratory for 6 days. Control group (n=5) is given placebo for a week and after week long oral dosing of each drug (n=5), animals arc sacrificed and following tissues are carefully taken out, weighed and stored at - 20°C for extraction of vasoactive substances.

1. Auricles
2. Right ventricle
3. Left ventricle
4. Aorta
5. Left carotid artery

6. Coronary artery.

NSAIDs are given according to following dose regimen.

Aspirin (120 mg/kg/day)	Aspro-Nicholas
Indomethacin (10mg/kg/day)	M.S.D. (Indocid)
Ibuprofen (120mg/kg/day)	Boots (Brufen)
Phenylbutazone (60mg/kg/day)	Cibe-Giegy (Butacoat)
Flurhiprofen (20mg/kg/day)	Boots (Froben)

During the oral dosing of drugs food and water 1s given ad-libitum to all groups of rabbits.

Extraction and Estimation Procedures

The method used for adrenaline and nor-adrenaline is that of Brownlee and Spriggs (1965) for estimation of SHT method of curzon and Green [(1970)] is employed. While method of shore, Bukhalter and Cohn (1959) is used to estimate the histamine.

All flourometeric estimation arc made on JASCO 550 Spectroflourometer details of the procedures of estimations are given elsewhere (Ahmed and Rasheed 1985).

Calculations

The level of significance (probability) is calculated by the method of student t-test as described by Bally in 1976.

Results

In present study, Aspirin and Indomethecin have produced significant reduction of adrenaline in auricles (Figure-1) while in ventricles all NSAIDs, except Indomathacin have produced very significant reduction of adrenaline (< .001).

Aspirin and Ibuprofen have reduced adrenaline contents in carotid and coronary artery and highest % of reduction is seen in Carotid artery i.e. 8% (<.001 Figure-1) while in aortra only Ibuprofen and flurbiprofen have produced very significant reduction (<.001). Aspirin and Indomethacin have raised adrenaline slightly in aorta and this rise in non-significant. Aspirin and Ibuprofen have reduced adrenaline contents significantly (<.001). Aspirin and Ibuprofen have also produced reduction of adrenaline in Coronary artery very significantly (<.001).

In auricles and ventricles all NSAIDs except Indomethacin have produced significant reduction of nor- adrenaline (< .001). Phenylbutazone, Ibuprofen and Hur-biprofen have also produced significant reduction (Figure-2).

Only Aspirin and Flubriprofen have shown significant reduction in Carotid artery (C.001), while in coronary artery except Aspirin all NSAIDs have failed to reduce nor-adrenaline.

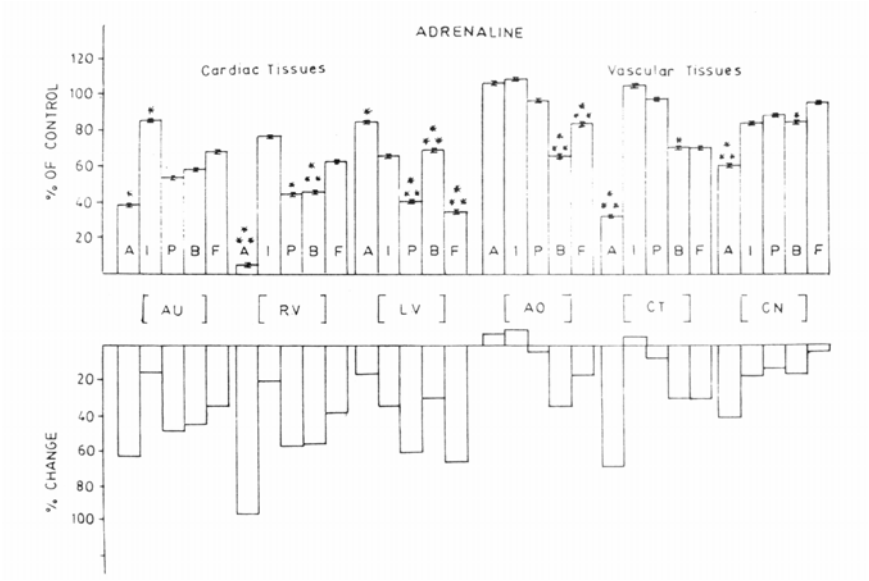

Figure-1: Histograms showing the percentage of control (Adrenaline) in Cardiovascular tissues. The percentage change is also shown.
Level of significance
* P.02
** P.01
*** P.001

Abbreviations

NSAIDS

A	=	Aspirin
I	=	Indomethacin
P	=	Phenylbutazone
B	=	Ibuprofen
F	=	Flurbiprofen

TISSUES

Au	=	Auricles
R.V.	=	Right ventricle
L.V	=	Left ventricle
AO	=	Aorta
CT	=	Left Carotid artery
CN	=	Coronary artery

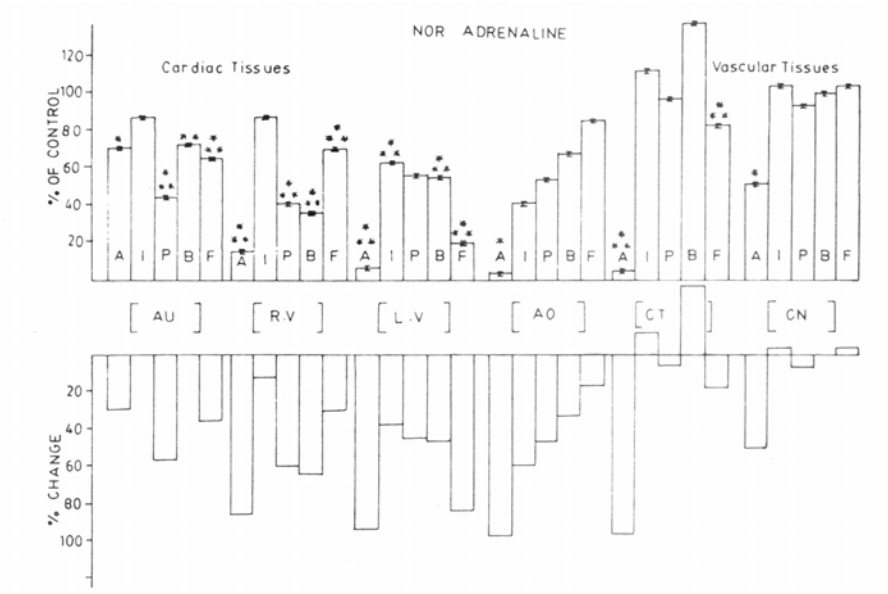

Figure-2 Histograms showing the percentage of control (Nor-adrenaline) in Cardiovascular tissues. The percentage change is also shown. Signs and levels of significance and abbreviations are explained in figure-1.

In Cardiac tissues all NSAIDs have failed to produce significant reduction of 5-HT (Figure-3). In left ventricle there is slight rise in 5-HT contents by Aspirin which is statistically non-significant.

Aspirin and Ibuprofen in comparison to other drugs have produced highest % of reduction i.e. 92% and 94% respectively (Figure-3). In carotid artery all NSAIDs have produced very significant reduction of 5-HT (<.001). In coronary artery Ibuprofen has demonstrated superiority over other drugs in reducing 5-HT (<.001). Aspirin and Phenylbutazone have produced non-significant reduction of 5-HT.

All of the NSAIDs have reduced histamine contents very significantly in auricle (<.001). In right ventricle except Phcnylbutazone all NSAIDs have produced significant reduction (Figure-4), while in left ventricle Ibuprofen and Indomethacin have failed to reduce histamine significantly. Aspirin, Phenylbutazone and Flurbiprofen have produced very significant reduction (< .001).

In vascular tissues all of the NSAIDs have failed to reduce histamine significantly (Figure-4) only Flurbiprofen has produced very significant reduction of histamine in coronary artery (<.001)

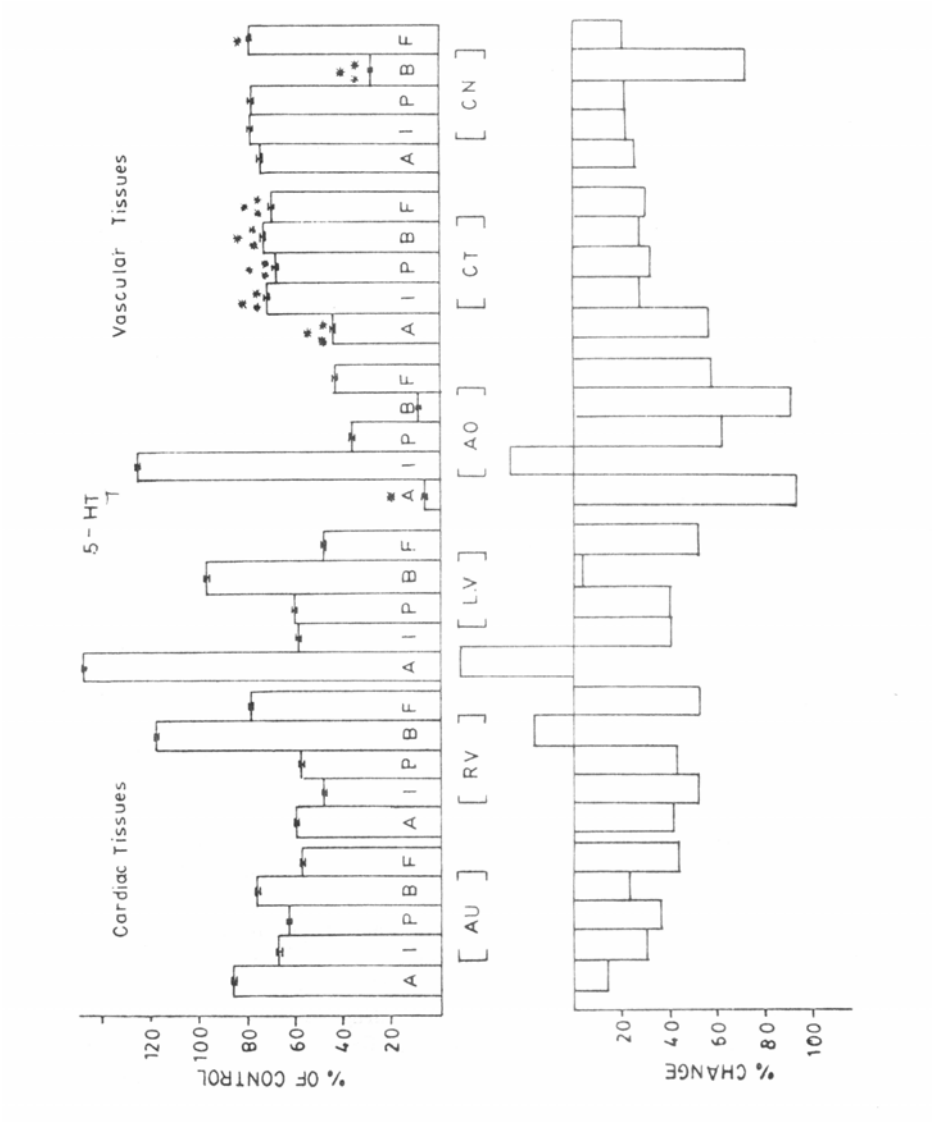

Figure-3 Histogram showing the percentage of control (5-HT) in Cardiovascular tissues. The percentage change is also shown. Signs and levels of significance and abbreviations are explained in figure-1.

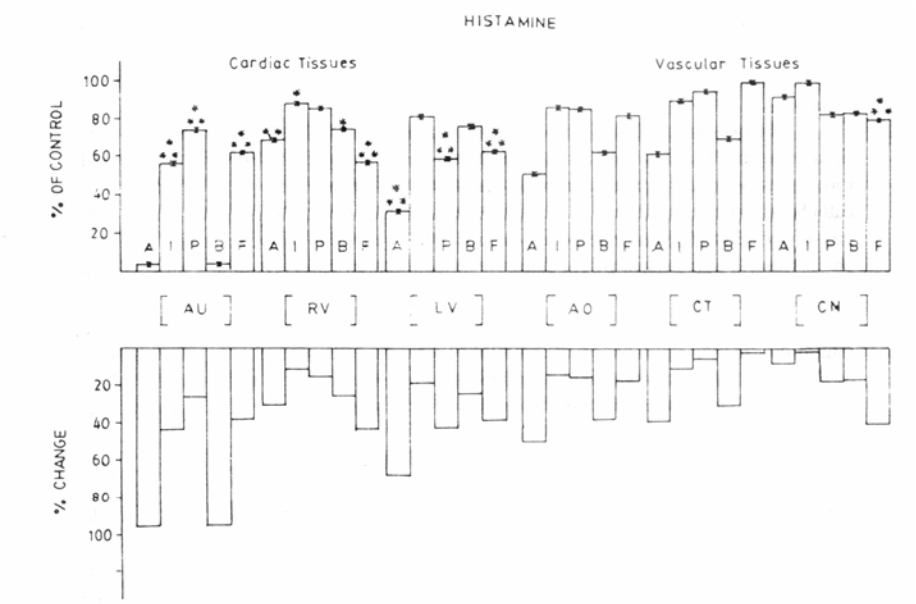

Figure-4 Histograms showing the percentage of control (Histamine) in Cardiovascular tissues. The percentage change is also shown. Signs and levels of significance and abbreviations are explained in figure-1.

Discussion

Non-Steroidal anti-inflammatory drugs are helived to act by competing directly with substrate for an active site on cyclo-oxygenase. Aspirin inactivates cyclooxygenase by acetylating serine residue of the enzyme, while lndomethacin and some other drugs inhibit cyclo-oxygenase by occupying some other site on the same enzyme (Roth & Siok 1978).

Inhibition of Prostaglandins alone is not sufficient enough to supress inflammation completely (Brune 1982). There are some other mediators of inflammation too as reported by Willoughby and Sedgwiek (1982). These mediators of inflammation (other than PGS) maybe 5-HT, histamine and catecholamines (klinc 1961: willoughby and Sedgwick 1982) with consideration of this above stated facts NSAIDs must now be explored for their mode of action and their capahilily of inhibinting all suspected and well known mediators of inflammation.

Present study has been designed to evaluate the effects of few well known and widely used NSAIDs on vasoactive substances which may be suspected of mediating and propagating inflammation on the basis of their nature and Pathophysiological effects.

In present study the vasoactive substances catecholamines are reduced very significanly by NSAID5 such as Aspirin , Ibuprofen Flurbiprofen, Phenylbutazone and Indomethacin (tool), which are in accordance of the results of Stjarne (1971), where catecholamines are reduced in tissues due to administration of NSAIDs Stjarne (1971) has reported that indomethhacin administration induced hyperesecretion of catecholamines in urine there by reducing tissue contents of catecholamines especially in heart.

Some of the NSAIDs like Flurbiprofen arc capable of inhibiting Leucocyte migration, platelet aggregation and inhibition of complete release of 5-HT (Yasunaga & Ryo 1975). Above mentioned mechanisms are responsible for enhancement of inflammation in cardiovascular system and lead to stimulated release of mediators of inflammation (Braunwald 1978; Willoughby and Sedgwick 1982).

Adrenaline induces platelet aggregation and stimulates release of thromboxane A2 (TxA2) which is a potent vasoconstrictor and mainly responsible for platelet aggregation (Braunwald 1978). Thus inhibition of adrenaline perhaps would lead to inhibition of TxA2 which could be a beneficial aspect in thrombotic diseases.

5-HT and histamine also have been reduced by these NSAIDs in some cardiac tissues and vessels. There are some supporting findings like the report of Barboni, voltatorni, Minelli, Polite and Materazzi (1977) demonstrating the inhibition of dopa decarboxylase by these drugs. This enzyme is responsible for the formation of above mentioned vasoactive substances. Inhibition of this enzyme may result in reduction of tissue contents of these substances, lessening the pathophysiologies of these substances and useful in treatment of hypertension.

Prostaglandins (PGS) interact with histamine in tissues and potentiates the inflammatory response of histamine (Willoughby & Sedgwick 1982). Inhibition of histamine may result in inhibition of histamine actions too, which would be useful in several inflammatory conditions of heart because the excess of histamine may cause arrythmias (Willoughby & Sedgwick 1982).

Inhibition of Ca^{++} uptake may effect the release of vasoactive substances (Diamont & Kruger, 1967; Northover, 1975), and NSAIDs effect cell membranes probably by inhibiting Ca^{++} uptake and stabilize cell membranes from lysosomal damage. NSAIDs also uncouple oxidative phosphorylation which may effect Ca^{++} evoked release of vasocative substances (Humphrey & Jaques, 1955; Rubin, 1969, 1970; Paulus & Whitehouse, 1973).

All of the above mentioned statement and reasons may be responsible for reduction of vasoactive agents by NSAIDs alongwith acting on PGS. NSAIDs probably act in most cases by preventing sensitization of pain receptors which may be stimulated by histamine, 5-HT and others, in the inflammatory arthritis process. These sub-stances also play a variable role in different types of inflammation (Hart & Huskisson, 1985).5-HT and Histamine cause vasodilation in some vascular beds, enhancing capillary

permeability, which is very typical feature of inflammation. These substances also cause pain and leucocyte emigration characteristic of inflammatory process. Some vasoactive substances probably play a small part in chronic arthritis (Hart & Huskisson, 1985).

As these vasoactive agents are involved in inflammatory conditions of heart, reduction of these could be beneficial in treatment of postmyocardial infarction pericarditis (P.M.I.P.), valvitis, phlebitis, post operative deep vain thrombosis, arrythmias and hypertension. Hyper aggregation of platelets by Adrenaline and 5-HT is well known (Elwood, 1983; Goodman and Gilman, 1985), and the reduction of these substances would reduce the chances of thrombosis.

Above mentioned drugs are anti-platelet drugs and may be used to prevent stroke (Fisher, Wiener, Cokene, and Cosine, 1984). NSAIDs mainly indomethacin and Flurbiprofen may be used to reduce pleurisy (Axel, Arviuer and Hakim, 1984). Combined administration of beta adrenoreceptor antagonists (Propranolol, Proctolol) and NSA/Ds like Aspirin and Indomethacin may be useful in reducing severity of arrythmias. Aspirin and Indomethacin reduce (phase 1B) arrythmias. Combined administration of B-adrenoreoptor antagonists may produce more pronounced antiarrythemic effects (Fagbemi, 1985).

By these findings of this presentation, it may be postulated that NSAIDs alongwith the inhibition of PGS may also reduce the inflammatory conditions by blocking the vasoactive substances which are the mediators of inflammation (Willoughby & WSedgwick, 1982).

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