

Effect of some non-steroidal anti-inflammatory drugs on ouabain-induced arrhythmias in guinea-pigs

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1 Effects of some non-steroidal anti-inflammatory drugs on ouabain-induced arrhythmias in guinea-pigs were studied.

2 Ventricular premature beats, ventricular fibrillation and cardiac arrest were induced in pentobarbitone-anaesthetized guinea-pigs by a slow intravenous infusion of ouabain.

3 Aspirin and indomethacin were found to accord a significant protection to the guinea-pigs against arrhythmias whereas ketoprofen was found to be ineffective.

4 It is concluded that the protective effect of aspirin and indomethacin may be due to inhibition of synthesis and release of thromboxane A₂ from the myocardium.

Introduction

The role of non-steroidal anti-inflammatory agents in cardiac arrhythmia and myocardial infarction has been of considerable interest during recent years. A clinical report in *Lancet* (Elwood & Sweetnam, 1979) showed that aspirin treatment significantly reduced the mortality rate in cases of myocardial infarction. Campbell *et al.* (1986), reported that in many clinical and experimental conditions non-steroidal anti-inflammatory agents like indomethacin, ibuprofen and piroxicam showed beneficial effects. Indomethacin was also reported to have a protective effect against myocardial scar formation after coronary artery occlusion in dogs (Jugdutt *et al.*, 1979; Hammerman *et al.*, 1983). Aspirin with its known anti-inflammatory and analgesic properties has also been reported to prevent ventricular arrhythmias in rats (Coker & Parratt, 1981) and dogs (Coker *et al.*, 1981; Fagbemi, 1984).

Thus it seemed worthwhile to study the effects of a few non-steroidal anti-inflammatory agents i.e. aspirin, indomethacin and ketoprofen, on the different stages of arrhythmia induced by ouabain in guinea-pigs.

Methods

Studies were carried out in albino guinea-pigs of either sex weighing between 350-450 g supplied by our Small Animal House. The method described by

Thomas & Tripathi (1986) was employed. The animals were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (60 mg kg⁻¹). The trachea was cannulated and positive pressure artificial respiration was maintained throughout the experiment by means of a rodent ventilator (Harvard Apparatus, England) at a rate of 45 strokes per min and the volume was adjusted to 1.0 ml 100 g⁻¹ body weight. The left common carotid artery was cannulated and connected to a Bentley-Trantec physiological pressure transducer for recording the blood pressure on a Gemini recorder (Ugo Basile, Model 7070). The right jugular vein was cannulated with a polythene tube and connected to a slow injection apparatus for infusion of ouabain. Limb lead II ECG was recorded on a Grass polygraph (Model 7-D) and heart rate was calculated from ECG signals. Ouabain solution (80 µg ml⁻¹) was continuously infused at the rate of 100 µl min⁻¹. The amount of ouabain required per 100 g body weight for the onset of early arrhythmia (indicated by appearance of ectopic beats, prolonged P-R intervals and P waves not followed by QRS wave), ventricular fibrillation and cardiac arrest was determined in control and drug-treated animals.

Drugs

The following drugs were used in this study: aspirin (IDPL), indomethacin (IDPL), ketoprofen (Elegan Pharmaceuticals, Italy) and arachidonic acid (Sigma). All the drugs except arachidonic acid were

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dissolved in 2% sodium bicarbonate solution and were injected intravenously; arachidonic acid was dissolved in 0.1% ethanol. Pretreatment time for indomethacin was 10 min; for aspirin and ketoprofen it was 20 min.

Statistical analysis

Students *t* test was used and the data are presented as mean \pm s.e.

Results

Effect on haemodynamic parameters

Aspirin, indomethacin and ketoprofen were without significant effect on the haemodynamic parameters. However, in general, a slight fall in blood pressure and reduction in heart rate was observed. The data are presented in Table 1. There was no change in ECG pattern and respiration rate. The hypotensive effect of arachidonic acid was significantly ($P < 0.05$) blocked by aspirin at a dose of 50 mg kg^{-1} and by indomethacin ($P < 0.05$) at a dose of 10 mg kg^{-1} ; however, ketoprofen at a dose of 20 mg kg^{-1} failed to produce any significant blockade ($P > 0.05$). The data are presented in Table 2.

Effect on ouabain-induced arrhythmia

Aspirin and indomethacin accorded a significant protection against ouabain-induced arrhythmia in guinea-pigs but ketoprofen did not (Table 3). The dose-response effect of all three drugs was studied but the anti-arrhythmic effect was not dose-dependent. The doses of ouabain required for the production of different stages of arrhythmia and cardiac arrest were increased in the drug-treated animals as compared to the untreated control animals; however, the degree of protection varied from one group to the other.

Table 2 Effect of some non-steroidal anti-inflammatory agents on hypotension induced by arachidonic acid in guinea-pigs

Group/drug	n	Fall in blood pressure (mmHg)		% inhibition
		Before treatment	After treatment	
Control	10	13 \pm 2.3	12 \pm 2.2	6
Aspirin (50 mg kg^{-1})	10	12 \pm 0.9	7 \pm 1.2	36*
Indomethacin (10 mg kg^{-1})	9	12 \pm 2.2	6 \pm 1.1	48*
Ketoprofen (20 mg kg^{-1})	9	11 \pm 1.9	11 \pm 2.0	—

* $P < 0.05$.

Discussion

The design of experiments in this study is of the type where an arrhythmogenic agent is infused until the death of the animal from ventricular fibrillation. The anti-arrhythmic drugs cannot prevent the death of the experimental animals and the effectiveness of anti-arrhythmic agent can only be demonstrated by the delay in the onset of different stages of arrhythmia and cardiac arrest in drug-treated groups.

In the present study, indomethacin and aspirin were found to antagonize the arachidonic acid-induced hypotension in guinea-pigs but ketoprofen was inactive. Indomethacin is reported to inhibit the cyclo-oxygenases responsible for the production of thromboxane A_2 and prostaglandins (Vane, 1976). Similarly aspirin has been found to inhibit the release of thromboxane A_2 in guinea-pigs (Piper, 1977).

Indomethacin and aspirin were anti-arrhythmic in guinea-pigs against ouabain-induced arrhythmia but ketoprofen was ineffective.

The mechanism by which non-steroidal anti-inflammatory agents are producing their anti-arrhythmic effect is not well established. It is clear from the results on the pharmacodynamic param-

Table 1 Effect of some non-steroidal anti-inflammatory agents on blood pressure and heart rate of anaesthetized guinea-pigs

Group	n	Blood pressure (mmHg)		Heart rate (beats min^{-1})	
		Before drug	After drug	Before drug	After drug
Control	33	57 \pm 1.4	53 \pm 1.6	282 \pm 6.5	263 \pm 6.5
Aspirin	22	54 \pm 2.0	52 \pm 1.7	263 \pm 6.7	242 \pm 7.4
Indomethacin	30	57 \pm 1.7	58 \pm 1.5	278 \pm 11.6	261 \pm 6.2
Ketoprofen	22	51 \pm 2.1	50 \pm 2.3	251 \pm 6.6	239 \pm 6.3

Values are mean \pm s.e. mean.

Table 3 Protective effect of aspirin, indomethacin and ketoprofen against ouabain-induced arrhythmias in guinea-pigs

Group	n	VEB	VF	CA
Control	40	18.08 ± 0.38	21.82 ± 0.58	29.72 ± 0.55
Aspirin 50 mg kg ⁻¹	16	19.57 ± 0.47*	25.44 ± 0.63***	31.61 ± 0.72*
Indomethacin 10 mg kg ⁻¹	18	21.39 ± 0.55***	28.23 ± 1.39***	35.25 ± 1.13***
Ketoprofen 20 mg kg ⁻¹	10	20.97 ± 1.51	24.63 ± 1.31	30.72 ± 1.03

Values are expressed as mean ± s.e. mean of the doses of ouabain required in $\mu\text{g } 100 \text{ g}^{-1}$ body weight to cause ventricular ectopic beats (VEB), ventricular fibrillation (VF) and cardiac arrest (CA).

* $P < 0.01$; *** $P < 0.001$.

eters that none of these agents has any significant direct effect on the heart rate and ECG pattern of the myocardium; hence, any direct antagonism by these agents on myocardium is ruled out. The other possible mechanism by which this effect could be obtained might be through the inhibition of prostaglandins and thromboxane A₂ synthesis and their release. It is well-established that non-steroidal anti-inflammatory agents inhibit the conversion of arachidonic acid into prostaglandins and thromboxane A₂ (Gryglewski, 1979). Inhibition of prostaglandin synthesis cannot be an anti-arrhythmic mechanism as this would facilitate the arrhythmia rather than prevent its appearance (Brasch, 1984). Cardiac thromboxane synthetases which are responsible for the production of thromboxane A₂ under hypoxic conditions are more sensitive and can be inhibited at a lower dose than that required for the inhibition of prostaglandins (Brasch, 1984). The cardiac glycosides are known to constrict the coronary arteries and induce a condition similar to that of ischaemia in ventricles (Fleckenstein & Byon, 1974; Fleckenstein *et al.*, 1979). Ischaemia leads to the formation and release of thromboxane A₂ (Coker *et al.*, 1981). It is reported that the coronary artery and other vessels contract *in vitro* after administration of synthetic thromboxane A₂ (Ellis *et al.*, 1976; Burke *et al.*, 1983; Coleman *et al.*, 1984).

In the present study, ketoprofen did not accord significant protection in guinea-pigs. Endogenous prostaglandins, PGF_{2a} and prostacyclin are known to be anti-arrhythmic (Coker & Parratt, 1981) and since ketoprofen is a potent inhibitor of the prostaglandin synthesis system (Tachizawa *et al.*, 1977; Shen, 1979), this effect of ketoprofen would prevent the synthesis of endogenous prostaglandins for their anti-arrhythmic effect. Indomethacin and aspirin were found to be anti-arrhythmic and might also be acting through some other mechanisms. Indomethacin is reported to be a calcium antagonist

(Northover, 1967) and a potent inhibitor of phosphodiesterase (Flores & Sharp, 1972) and thromboxane (Colasanti & Ernst, 1978). Aspirin is also reported to have a selective platelet cyclo-oxygenase inhibiting effect (Smith & Willis, 1971; Korbut & Moncada, 1978), thus preventing the synthesis of thromboxane A₂. Coker *et al.* (1981) found that aspirin inhibits the formation and release of thromboxane A₂ and thereby prevents the occurrence of ventricular ectopic beats in coronary artery ligated dogs. A positive correlation was established between the release of thromboxane A₂ and occurrence of ventricular ectopic beats.

It is generally accepted that thromboxane A₂ may contribute to the pathogenesis of a variety of vascular disorders such as myocardial infarction, angina pectoris, thrombosis, circulatory shock and sudden death (Shimamoto *et al.*, 1979; Walinsky *et al.*, 1984; Darius *et al.*, 1985; Lefer, 1985). A thromboxane A₂ antagonist is reported to have an anti-arrhythmic effect in dogs (Coker & Parratt, 1984). Parratt & Wainwright (1986) have also supported the role of thromboxane A₂ in the genesis of arrhythmia and suggested that the blockade of thromboxane A₂ receptors may be a suitable protective approach to anti-arrhythmic therapy.

Thus, it is concluded from the above findings that the non-steroidal anti-inflammatory agents showing anti-arrhythmic effect in guinea-pigs against ouabain-induced arrhythmia in the present study may be mediating their effects through inhibition of synthesis and release of thromboxane A₂.

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