Pharmacology Biochemistry & Behavior, Vol. 10, pp. 285–291. Printed in the U.S.A.

The Effect of Sulfinpyrazone on Morphological Changes in the Coronary Vasculature Induced by Prolonged Unpredictable Stress in the Rat

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(Received 11 August 1978)

CAIRNCROSS, K. D., J. R. BASSETT AND C. MARTIN. *The effect of sulfinpyrazone on morphological changes in the coronary vasculature induced by prolonged unpredictable stress in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 285–291, 1979.—It was confirmed that prolonged unpredictable stress in the rat induces morphological change in the coronary microcirculation. These changes include dilation in venules, deposits staining positive with PAS in the venules due to platelet aggregation and a breakdown of the endothelial lining in arterioles. Sulfinpyrazone is reported to prevent platelet agglutination, and shows effectiveness in the clinic in preventing re-infarction following infarction. Accordingly rats were exposed to the stress regimen for 50 days, and groups were treated with sulfinpyrazone, either prophylactically (receive drug for 50 days) or therapeutically (receive drug Day 30 to Day 50). The morphology of the hearts of treated animals were compared with those of placebo treated controls. It was demonstrated that therapeutic sulfinpyrazone did not prevent ($p<0.01$), but reduced the incidence of morphological change in the coronary microcirculation. Prophylactic sulfinpyrazone had a distinct protective effect ($p<0.001$). It was demonstrated that the plasma corticosterone levels in both drug groups did not fall to the level found in control groups. The results are discussed in terms of a glucocorticoid-sulfinpyrazone interaction preventing prostaglandin release which will prevent platelet aggregation. It is possible that the interaction relates to maintaining the integrity of the microcirculatory endothelial cells, thus preventing the local release of inflammatory substances.

Sulfinpyrazone Coronary vasculature Unpredictable stress

EXPOSURE of the rat to prolonged stress (70 days) in which the psychological parameters of anxiety were evident induced morphological change in the coronary microcirculation. The changes included congestion and dilation in venules, collecting venules and veins, vacuolation in the intima-medial layers of the coronary arterioles, marked mast-cell infiltration into coronary peri-vascular areas, and platelet aggregation within the coronary microcirculation [2]. This was confirmed in an ultrastructural study which demonstrated the presence of junctional gaps in the endothelial lining of the capillaries as well as platelet aggregation and platelet adherence to the capillary wall [3].

These morphological changes occur some 40 days after the initiation of unpredictable stress, at a time when the plasma corticosterone level drops from the extreme level (circa 90 μg/100 ml blood plasma) to a moderate elevation (circa 40 μg/100 ml) [3]. This latter observation offers a basis for the development of an in vivo animal model to examine the effectiveness of drugs which have clinical potential in the treatment of coronary vascular disease. One such drug is sulfinpyrazone (1,2-diphenyl-4-2′ phenylsulfinethyl -3,5 pyrazolidinedione), which has been demonstrated to inhibit platelet aggregation both in vitro and in vivo [4,14].

Accordingly it was decided to conduct a long term experiment in mature rats, in which the animals would be subjected to the unpredictable stress regimen previously shown to induce morphological changes in the coronary microcirculation. Further, as these vascular changes occur when the circulating corticosterone level has dropped to moderate levels, it was decided to treat the animals in two ways. Firstly, animals would be treated with sulfinpyrazone throughout the whole of the experiment, to examine the premise that the drug might have a prophylactic effect in preventing morphological change. Secondly, the animals would be treated with sulfinpyrazone after 30 days of unpredictable stress, to examine the therapeutic effect of the drug on the morphological changes which by that time would have been initiated.

METHOD

Male CSF rats 116–121 days old were used in all experiments. The animals were housed in groups of 3 under conditions of constant temperature and humidity (21 ± 0.5°C, 46% humidity) and subjected to a reverse light schedule (light 8 p.m.–8 a.m.) beginning 15 days prior to the commencement of experimentation and continuing until conclusion. Food and water were provided ad lib.

Drug Treatment

The animals were randomly assigned to four groups (12 animals/group). Group 1 (PS) received sulfinpyrazone (8 mg/kg) daily for the duration of the experiment (prophylactic group). Group 2 (TS) received sulfinpyrazone (8 mg/kg) after

30 days of the stress regimen (therapeutic group). Group 3 (PO) received no drug, but 2 ml peanut oil as the drug vehicle. Group 4 (S) received no oral injection, but were stressed for the duration of the experiment. Each group consisted of 12 animals, and the experiment had a duration of 50 days.

Stress Apparatus

Animals were placed in automated one-way avoidance boxes (Lafayette Model No. 85200). An escape platform was made available to the animal by an automated movable partition. A light conditioned stimulus (CS) of 2 W was located on the wall of the grid chamber opposite to the escape platform. The unconditioned stimulus (UCS) was delivered by a generator-scrambler through the grids as a 2 mA, 50 pulses/sec square wave. Each rat was placed on the escape platform at the commencement of the treatment session. On each trial the CS onset 4 sec before the animal was pushed by the movable partition from the platform onto the grid which was simultaneous with the onset of the UCS. At this point in time the movable partition immediately retracted and the animal was able to jump from the grid to the re-exposed platform with a minimum latency of 0.3 sec. The UCS was terminated by the return of the animal to the platform.

Stress Procedure

The stress treatment consisted of 7 CS–UCS exposures randomly placed in a 35 min stress session. Each stress session was repeated daily Monday–Friday. Stress treatments were carried out between 9 a.m.–12 noon. Immediately following the completion of the last stress episode the animals were sacrificed by cervical dislocation and exsanguinated. The blood was collected in heparinized tubes and centrifuged in order to obtain cell free plasma which was then frozen and stored at –20°C. Plasma corticosterone levels were determined by the fluorimetric method of Mattingly [9] which is specific for free 11-hydroxycorticosteroids.

Histology

Specimens were fixed in 10% formal saline for 7 days then paraffin embedded. Transverse sections 7 μ thick were cut from the heart starting at the apex. The sections were stained with haematoxylin (Harris) and eosin (H and E), or were diastase treated to remove glycogen and stained with periodic acid fuschin and counterstained with Weigert's iron haematoxylin (PAS); alternating 6 sections of each stain. Body weight: Animals were weighed daily for three days prior to the commencement of the experiment and daily during the course of the experiment. This to ensure that the drug regimen, together with daily injections and unpredictable stress did not produce untoward changes in the animals' well being. Histological evaluation was undertaken by one investigator working on a blind basis with respect to treatment of animals. Five slides were evaluated from every heart, moving from the apex to the left ventricular region. The slides were scored according to an arbitrary numerical system, the criteria of which is set out in Table 1. A rank was assigned to each slide in every group (S, PO, PS, TS), and the results subjected to a Mann-Whitney U test (n=12).

TABLE 1

Score	
0	Normal appearance of venules.
2	Dilatation of venules without the appearance of PAS positive staining deposits.
4	Dilation of venules with the appearance of PAS positive staining deposits marginating the endothelium of the venule.
6	Dilation of venules, PAS positive deposits within the lumen of the vessel and PAS positive deposits in perivascular areas.
8	Extensive dilation of venous radicles. PAS positive material evident in all fields examined (×80). Evidence of fibrin deposition in perivascular areas and vacuolation of arterioles.

RESULTS

Biostatistical analysis of the results obtained in this study indicate no difference between S, and PO, groups with regard to morphological change in the coronary microcirculation. However, using the criteria previously described (Table 1), a significant statistical difference was evident between the control groups (S and PO) and animals treated with therapeutic sulfinpyrazone (TS, $p<0.01$). An even greater significant difference was demonstrated between control animals (S, and PO) and those treated with prophylactic sulfinpyrazone (PS, $p<0.001$).

Histological examination of hearts taken from 50 day stressed animals show the pathological changes described in our previous study [2], and are illustrated in Fig. 1. There exists a pronounced degree of congestion and dilation of the micro-circulation particularly in the venules and collecting venules. This was evident in the apex of the hearts and in the left ventricular wall. Associated with the venous congestion, there occurred the deposition of PAS positive material marginated in the venous endothelium, particularly in the dilated vessels. These morphological changes were observed in every animal of the stressed control group (S) and in the animals treated with peanut oil alone (PO). Additionally, there occurred changes in the coronary arteriolar system. These, as previously described, included oedema and vacuolation of the intima-media.

The morphological changes described although evident, did not occur with the same frequency in animals treated with sulfinpyrazone therapeutically ($p<0.01$). Thus, in Fig. 2 is evidence of deposition of material that will stain PASive marginated in a venule. The higher magnification used in Fig. 2 allows capillary distension to be observed also. It was noted that in the therapeutic group venular distension occurred more to the periphery of the ventricular muscle, small Thebesian veins did not show evidence of congestion. In Fig. 3 is illustrated the result of treating rats with sulfinpyrazone prophylactically. It can be seen that venular distension is minimal, and that there is little evidence of PASive material marginated in the endothelium ($p<0.001$).

Sulfinpyrazone treated rats in both the prophylactic and therapeutic groups did not show evidence of the prevalence of arteriolar degeneration found in the untreated stress groups. In general arterioles had intact intima and no evidence of vacuolation of the media.

Weight Gain

Mature rats 116–121 days of age at the outset of the exper-

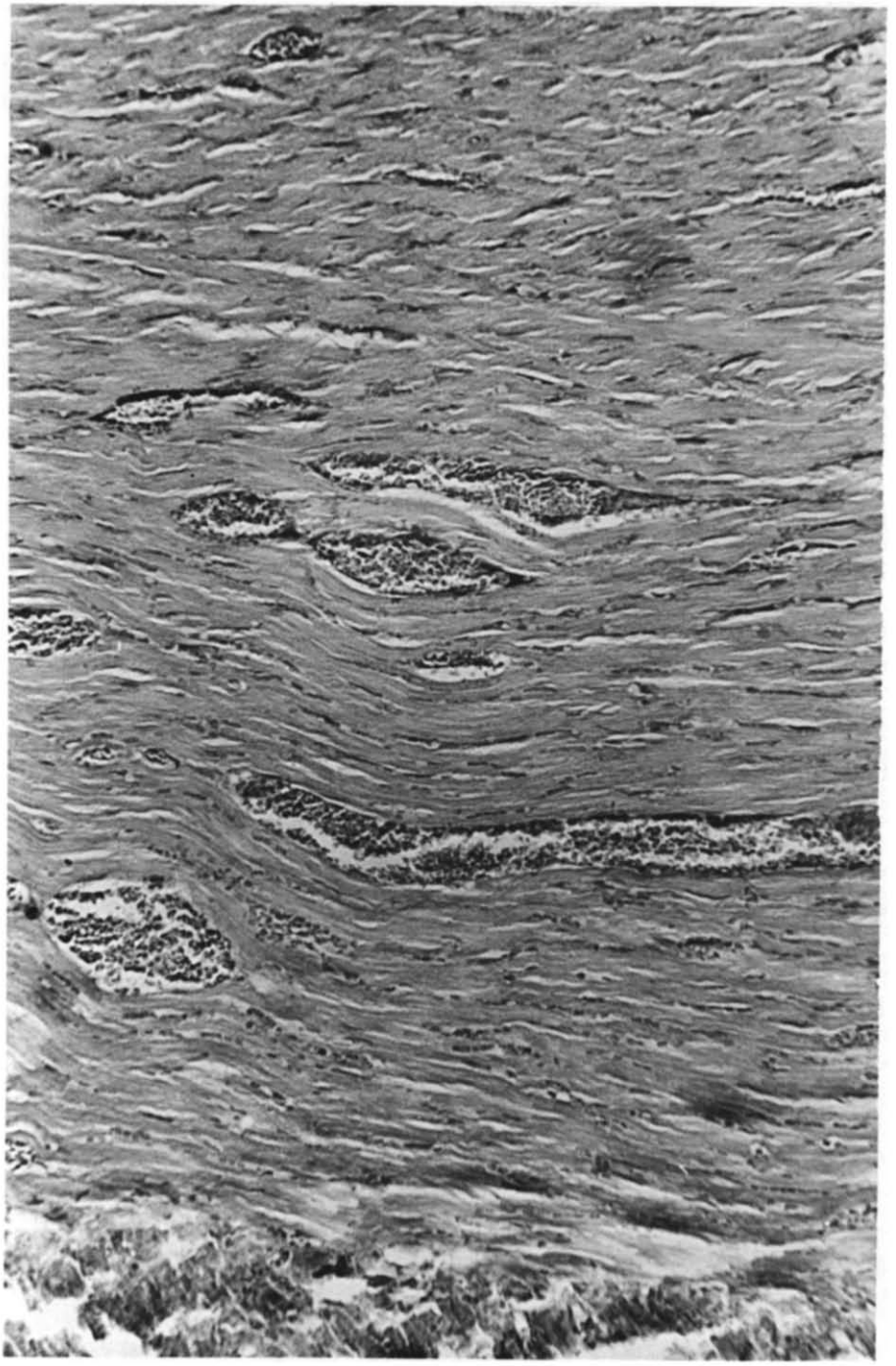

FIG. 1. Extensive dilation in venous radicles in hearts from 50 day stress animals without drug treatment, H. and E. (×80). (Mann-Whitney scale—8).

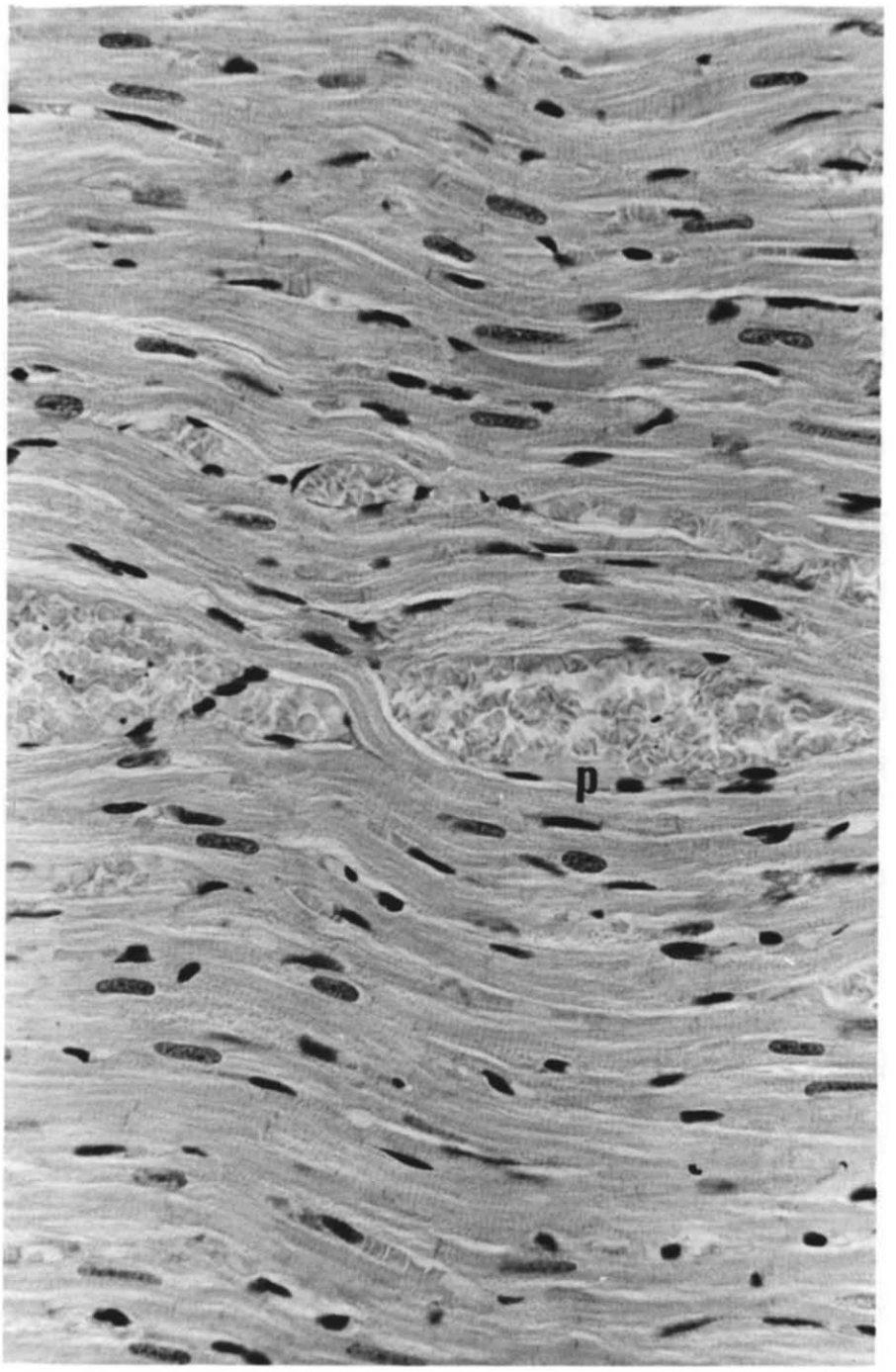

FIG. 2. Dilatation in venule of rat treated with therapeutic sulfinpyrazone. Note all venules do not show a similar degree of dilation. H. and E. (×130). P=deposition of material that will stain PAS+ve marginating the endothelium of venule (Mann-Whitney scale—4).

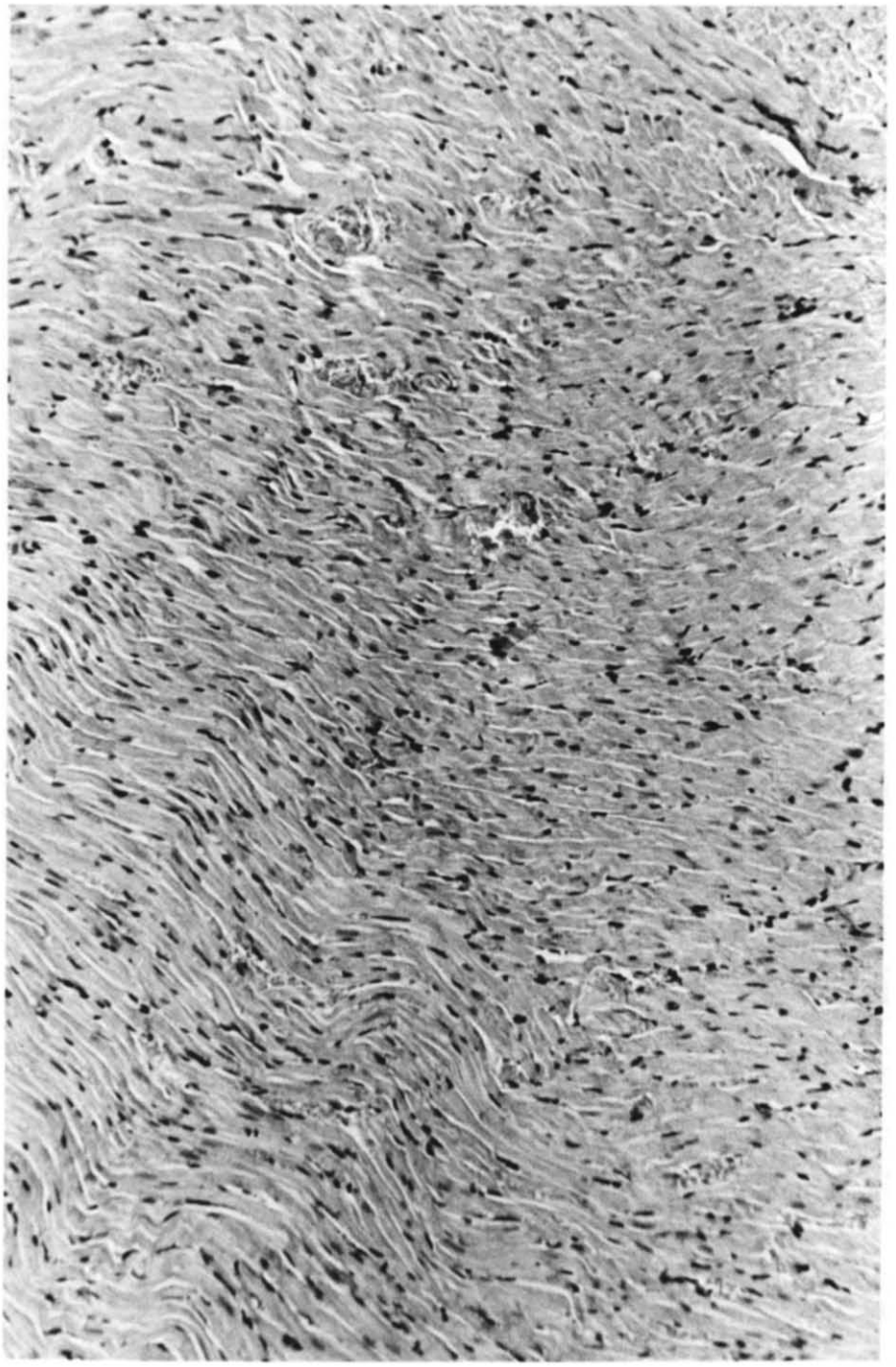

FIG. 3. Normal appearance of venules in rats treated with prophylactic sulfinpyrazone. H. and E. (×80). (Mann-Whitney scale—0).

iment weighed between 503 and 519 g—on average, and at the end of the experiment there was no statistically significant difference between weight gain in any group. It can be assumed therefore that the experimental drug regimen did not adversely affect any of the four groups.

Plasma corticosterone response. The mean plasma 11-hydroxy-corticosterone levels (in μg/100 ml blood plasma $\pm$ SE) on Day 50 of the experiment were for the C group 47.7 $\pm$ 4.2, and for the PO group 48.5 $\pm$ 4.6. This difference is not significant using a *t*-test, and corresponds to the moderate steroid elevation found by Bassett and Cairncross [2] in previous experiments. In the case of the PS and TS groups the glucocorticoid levels were 69.9 $\pm$ 3.4 and 62.6 $\pm$ 5.6, respectively. Again, these levels were not significant from each other, but the levels were significant ($p<0.05$) when the PS and TS groups were compared with the PO and S groups, respectively.

DISCUSSION

Sulfinpyrazone has been shown to be effective in reducing the degree of morphological change in the coronary microcirculation in the rat following prolonged unpredictable stress. Such a conclusion is important for two reasons. First it reinforces recent clinical investigation regarding the effectiveness of sulfinpyrazone in preventing cardiac death after myocardial infarction [15]. Secondly, it confirms that the animal model previously described by Bassett and Cairncross [2,3] can be used to study the etiology and therapy of coronary vascular disease. In discussing the development of the pathology following unpredictable stress, these authors observed that the coronary microcirculatory changes did not occur before 20 days of stress and were evident by Day 40. It was during this period that a physiological adaptation occurred in that circulating glucocorticoid levels fell from the extreme level of circa 90 μg/100 ml plasma to moderate levels (circa 40 μg/100 ml). The inference being that extreme steroid elevation has a protective action on the development of morphological degenerative change in the coronary microcirculation. Following the phase of adaptation there occurs a reduction in the hyperactivity of the hypothalamic-hypophyseal-adrenocortical axis, producing a fall in ACTH output and hence glucocorticoid secretion [11].

The glucocorticoids have a protective action against the effects of endogenous inflammatory substances, preventing passage of macromolecules across the endothelial lining of the coronary microcirculation into the media of coronary arterioles or into perivascular areas in the capillary bed [6,13]. The question of how this is achieved must be considered. The adaptive response to an endogenously induced inflammatory challenge which produces vascular leakage by opening endothelial gaps results in an attempt to close such gaps, an effect achieved by platelet aggregation, and an effect described and demonstrated by Bassett and Cairncross [3]. Evidence has been presented recently that there exist in the vasculature two prostaglandins which play an integral part in platelet response to microcirculatory change. These are prostaglandin PGX which prevents platelet aggregation and platelet adherence to the coronary microcirculatory wall [10]. PGX, or prostacyclin has a main function which is "to protect the coronary circulation against the formation of blood platelet aggregates, and therefore preventing occlusion of small branches of the vulnerable coronary tree" [5]. However, damage to the vascular endothelium results in the release by platelets of a second prostaglandin—thromboxane A_2 which will itself, induce platelet aggregation in those areas where endothelial damage is manifest [10]

It has been reported that high levels of glucocorticoids will prevent prostaglandin release, but not their synthesis [7]. Thus, a situation can be envisaged whereby potential morphological damage in the coronary microcirculation, although overtly possible, is prevented by glucocorticoid action on the cellular interface. Additionally it must be considered that the coronary endothelial cells possess the enzymatic means of synthesising histamine [13]. Such a histamine source must subserve a physiologically adaptive function. It would appear that the release of histamine by damaged endothelial cells of the coronary microcirculation acts as the stimulus inducing platelet aggregation to plug endothelial gaps and prevent the passage of macromolecules from the vascular system to extravascular deposition.

Such a view is substantiated by the work of Sherry *et al.* [15] who suggest that platelet emboli become released into the coronary microcirculation and produce occlusions which result in often fatal arhyrthmia. The point is made that the clinical literature in describing the sequalae of platelet emboli in the coronary microcirculation suggest a process which follows closely the sequence of morphological and ultrastructural change described by Bassett and Cairncross following exposure of the rat to prolonged unpredictable stress [2,3].

The experimental results described in this presentation indicate that the endothelial damage which normally occurs in the coronary arterioles following unpredictable stress did not occur when sulfinpyrazone was administered prophylactically, and was reduced in the therapeutic sulfinpyrazone group. Further, the venular dilation so characteristic of the untreated unpredictable stress group is noticeably absent in the PS group, and the incidence of PAS-ive deposits in the TS group, although occurring is of reduced indicence. It is instructive to examine the pharmacological properties of sulfinpyrazone in terms of these experimental observations.

Sulfinpyrazone has the ability to lengthen platelet survival and decrease platelet turnover in the clinical situation in patients suffering from thromboembolitic disorders [14]. The basis for this clinical observation remains non-delineated, although experimental studies, both in vivo and in vitro have shown sulfinpyrazone to inhibit platelet aggregation [4]. Other studies have shown that this drug is a potent inhibitor of prostaglandin synthesis [1]. It is pertinent that the glucocorticoids also inhibit prostaglandin release. It is pertinent also, that in the presence of long term sulfinpyrazone administration in the experimental model, glucocorticoid plasma levels do not fall to the moderate elevation observed in the two control groups. Thus, a situation exists in the model whereby sulfinpyrazone, an exogenously administered drug, and endogenous glucocorticoids have an apparent complementary effect in terms of an inhibition of prostaglandin release and in preventing platelet agglutination. The immediate question arising from such an observation is why glucocorticoid plasma levels should remain elevated in long term sulfinpyrazone administration, when in other circumstances they would fall to moderate levels. A further question, is the glucocorticoid sustaining effect a reason for the demonstrated clinical efficacy of sulfinpyrazone in thromboembolytic disorders?

In part answer to this last question, it may be argued that although the immediately demonstratable actions of both

sulfinpyrazone and the glucocorticoids is one of prevention of platelet agglutination, such an effect could not be achieved if the integrity of the coronary vascular endothelium were not preserved, an effect clearly demonstrated in the present study. This observation receives support from the recent work of Harker *et al.* [8], who demonstrated both in vivo and in vitro, vascular endothelial protection by sulfinpyrazone. Further, the in vivo studies described were conducted over a three month period [8] a time period exceeding the 50 days described in the current experiment. Thus, it may well be argued that the efficacy of a sulfinpyrazone-glucocorticoid interaction does not wholly relate to an anti-platelet agglutination action, but to an action preserving the coronary vascular endothelium. Such an effect might prevent activation of the platelet agglutinin, thromboxane A_2, and help protect the coronary microcirculation against degenerative change. Such a situation is currently examined.

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