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Age and the antihypertensive effect of aspirin in rats

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1 We previously showed that chronic exposure to aspirin (100 mg kg^{-1} daily, by mouth) is effective in preventing the onset of hypertension in young (28–84 day old) spontaneously hypertensive rats (SHRs). This is contrary to what others have reported using older SHRs.

2 Renal prostaglandin $F_{2\alpha}$ was also reduced in young SHRs and Wistar-Okamoto strain rats (WKYs) exposed to aspirin.

3 In the present study we extended the period of aspirin treatment in young rats to beyond 84 days of age. We found that aspirin lost its antihypertensive effect in SHR and WKY rats at 110 ± 7 days of age regardless of whether the exposure to aspirin had begun at age 28, 49 or 87 days.

4 We conclude that the loss of antihypertensive effect of aspirin in the SHR and in older WKY rats, is determined by some factor(s) probably not related to prostaglandin $F_{2\alpha}$, which reaches full expression in the 110 ± 7 day old rat, or is fully dissipated at this age.

5 The anti-$PGF_{2\alpha}$ activity of aspirin in the SHR and WKY rat was short-lived and apparently unrelated in time to the antihypertensive effect of aspirin.

Introduction

Aspirin, the archetype cyclo-oxygenase inhibitor, has no antihypertensive effect in the adult spontaneous hypertensive rat (SHR), or in man (Ferreira & Vane, 1967; Chrysant *et al.*, 1980). Indeed, the contrary is more often true; aspirin may cause blood pressure and peripheral vascular resistance to increase (Scholkens & Steinbach, 1975) possibly because of a decreased synthesis of prostaglandin E_2 (PGE_2), a recognized modulator of vasodilator activity in its own right (Riesterer & Jaques, 1968). However, in the prepubertal SHR, we have recently shown that aspirin by mouth (100 mg kg^{-1}, daily), given chronically from 28 to 84 days of age, can prevent the development of hypertension in the SHR, an action which was associated at 84 days by a decrease in renal prostaglandin E_2 and $F_{2\alpha}$ (Tuttle *et al.*, 1985b). The present study was designed to determine if aspirin had an antihypertensive effect in rats older than 84 days of age and, if not, whether the age of the rat, or the duration of exposure to aspirin was the critical determinant in this loss of the antihypertensive effect. Renal PGF_2 activity was determined in each protocol as the most likely moiety to be associated with the antihypertensive effect of aspirin.

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Methods

One hundred and forty-four male, 21 day old SHRs and an equal number of normotensive controls, WKYs (Wistar-Okamoto strain, Taconic Farms, Germantown, NY), were divided into 6 equal groups. Systolic blood pressures were determined weekly in all rats by tail cuff technique using an electronically assisted plethysmograph (Technilab, Pequanock, NJ). Rats had blood pressures recorded while resting quietly in an acrylic chamber at 35°C. Pilot studies, using a direct intracarotid method for recording of pressure, were done randomly on rats just before they were killed for PGF_2 analysis in order to monitor the tail cuff method. We noted no differences in excess of ±9%. Water consumption was controlled and monitored by checking the volume and frequency of replacement of the drinking vessels. In a previous study we noted that older SHRs were generally lighter than the WKYs but their food and water consumption was not different. Neither did we find evidence of occult blood or reduced haematocrits in aspirin-treated rats (Tuttle *et al.*, 1985a, b).

Drug protocol

At the end of the first week, the first group of 24 WKY and 24 SHR rats, now 28 days old, were sup-

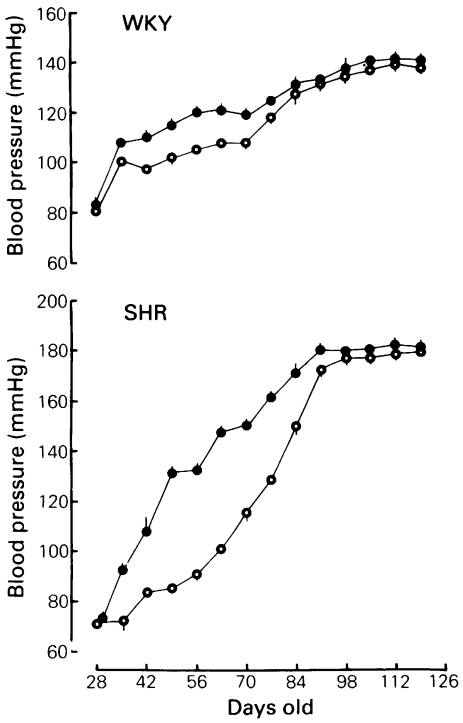

Figure 1 Effect of aspirin (100 mg kg^{-1} daily, by mouth) on mean systolic blood pressure of SHR and WKY rats from the 28th to 119th day of age: (●) controls receiving plain water; (○) rats receiving water plus aspirin. Each point represents the mean of 6–8 rats; s.e. shown by vertical lines.

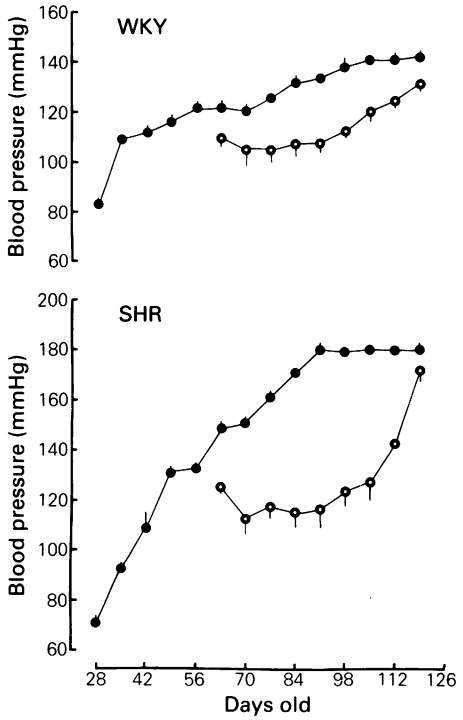

Figure 2 Effect of aspirin (100 mg kg^{-1} daily, by mouth) on mean systolic blood pressure of SHR and WKY rats from the 49th to 119th day of age: (●) controls receiving plain water; (○) rats receiving water plus aspirin. Each point represents the mean of 6–8 rats; s.e. shown by vertical lines.

plied with water *ad libitum* containing aspirin (1.0 mg ml^{-1}) achieving a dose level of 80–100 mg kg^{-1} per day. The second and third groups of rats received no aspirin by mouth until the 49th and 87th day of age respectively. Each of the 3 groups of SHR and WKY rats outlined in the aspirin protocol were accompanied by 3 groups of equal size and age that received no drug. These served as controls and were subjected to the same protocols and testing as the drug-treated rats. Blood pressure and heart rates were recorded weekly in each group of rats. Depending on the protocol, rats were killed at 28, 56, 98, and 126 days of age for prostaglandin F_2 analysis by radioimmunoassay of ethylacetate extracted, quick frozen, renal cortical tissue homogenates by methods described in detail by Tuttle *et al.* (1985a).

Statistics

Blood pressures and renal PGF_2 values were pooled within groups, by age and treatment protocols, and averaged (±s.e.). Statistical comparisons between same age groups and between groups representing different ages and protocols were made using an inhouse ANOVA programme. Statistical significance was set at the $P < 0.01$ level with n ranging from 5–8.

Results

Blood pressure

The antihypertensive effect of aspirin was lost when SHRs and WKYs reached 110 (±7) days of age. This loss of effect was independent of the age at which exposure to aspirin had begun. This is shown in Figures 1–3 where groups of rats (SHR and WKY) received aspirin first at 28, 49, and 87 days of age respectively. It is no less remarkable that, although the developing state of hypertension in the older WKYs was much less than in the SHRs at the same age, similar effects of aspirin were seen in the WKYs

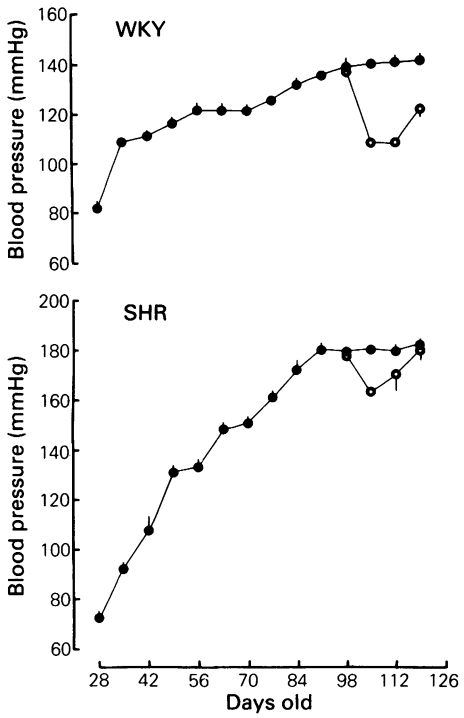

Figure 3 Effect of aspirin (100 mg kg^{-1} daily, by mouth) on mean systolic blood pressure of SHR and WKY rats from the 87th to 119th day of age: (●) controls receiving plain water; (○) rats receiving water plus aspirin. Each point represents the mean of 6–8 rats; s.e. shown by vertical lines.

as in the SHRs. The dynamics of the loss of the antihypertensive effects of aspirin and return to high blood pressure in both the SHRs and WKYs depended upon the age at which aspirin ingestion had begun. Recovery occurred slowly for rats receiving aspirin at a young, prehypertensive age and rapidly for rats first receiving aspirin at older hypertensive ages.

Prostaglandin $F_{2\alpha}$

Young, SHRs killed at 28, 56, and 98 days of age had higher renal $PGF_{2\alpha}$ activities than similarly aged WKYs (Table 1). The ability of aspirin to effect a change in these values was sharply limited by the duration of ingestion. Thus only in rats receiving the drug for periods of 28 days or less was renal $PGF_{2\alpha}$ lower than in the control. This is dramatically shown by comparing the mid and endpoint values of the 98–126 day group with those of the preceding groups.

Discussion

The salicylates and salicylate-like agents project a spectrum of activities in living systems, many of which are based upon their anti-cyclo-oxygenase activities, such as their prolongation of blood clotting times (Campbell *et al.*, 1982) and the blockade of the diuretic or antihypertensive effects of some drugs (Riesterer & Jaques, 1968; Durao *et al.*, 1977; Imai *et al.*, 1983). Recent evidence (Tuttle *et al.*, 1985a, b), which is confirmed in the present study, shows that aspirin can prevent and reverse a state of hypertension in the young SHR and WKY rat. We, like Armstrong *et al.* (1976), have also detected elevated levels of $PGF_{2\alpha}$ in the prehypertensive SHR. Attempts by others to reduce blood pressure in adult hypertensive subjects, either man or animal, by using aspirin or its counterparts, have not been successful

Table 1 Prostaglandin $F_{2\alpha}$ (μg 100 g^{-1} renal cortex) in SHR and WKY rats at the beginning, midway and end of chronic ingestion of aspirin (100 mg kg^{-1} by mouth)

Group	*Treatment*	*Duration in days old*	*Starting $PGF_{2\alpha}$*	*Midway $PGF_{2\alpha}$*	*Ending $PGF_{2\alpha}$*
WKY	None	28–126	32 ± 6.1	31 ± 4.0	33 ± 6.2
WKY	Aspirin	28–126	*	44 ± 2.5	39 ± 5.0
SHR	None	28–126	53 ± 1.0	†79 ± 8.0	43 ± 3.0
SHR	Aspirin	28–126		54 ± 3.7*	47 ± 2.6
WKY	None	56–126	31 ± 4.0	21 ± 1.2	32 ± 1.9
WKY	Aspirin	56–126	*	20 ± 6.0	35 ± 2.2
SHR	None	56–126	79 ± 8.0	†34 ± 3.5	†42 ± 2.0
SHR	Aspirin	56–126		†42 ± 2.7	†45 ± 2.4
WKY	None	98–126	21 ± 1.2	28 ± 2.6	†62 ± 4.0
WKY	Aspirin	98–126	*	†11 ± 0.8*	†40 ± 4.7*
SHR	None	98–126	34 ± 3.5	41 ± 0.5	†64 ± 4.4
SHR	Aspirin	98–126		27 ± 7.0*	44 ± 4.0*

Significance: $P < 0.01$ between starting value (†): between pairs (*).

(Chrysant et al., 1978; Quilley et al., 1987). Our present studies suggest that indeed in the older rat aspirin has a shorter and less antihypertensive effect than in the young rat. Although the WKY animals lagged somewhat behind SHRs in demonstrating this loss of effect of aspirin, this trend was clearly evident in the WKYS at 110–126 days of age.

These data raise the question as to whether the loss of antihypertensive activity in the older SHR and WKY is related to a loss of some activity of aspirin, or to an age-related change in the older rat's susceptibility to aspirin.

Physiochemical, or developmental, adaptation to the presence of an inhibitor, or poison, is a common explanation given to explain why the effects of a number of drugs or cellular poisons diminish with time. We attempted to test this possibility in the present study by 'tracking' one factor, $PGF_{2\alpha}$ through the study since anti-cyclo-oxygenase activity is the most commonly cited biochemical effect of aspirin. We found that not only was the anti-cyclo-oxygenase activity lost within 2–3 weeks of treatment with aspirin, but even when it was present, it bore no relationship to the status of the effect of aspirin on blood pressure. Thus aspirin reduced $PGF_{2\alpha}$ in the 98–126 day treatment group but at the same time its antihypertensive effect was diminishing.

Recent studies (Quilley et al., 1987), seem to show that when urinary prostaglandin excretion is used as a measure of synthesis in the SHR, the effect of aspirin may be limited to a span of days rather than of weeks. Our data tend to support this conclusion. It is unlikely then, due to the relatively long duration of the antihypertensive effect on one hand and short duration of anti-cyclo-oxygenase of aspirin on the other in the young SHR, that re-emergence of $PGF_{2\alpha}$ can be used as a mechanism to explain the re-emergence of hypertension.

There are of course, other aspirin-sensitive mechanisms besides those concerned with the prostaglandins, but if adaptation by them occurs over time of exposure to aspirin, shortening the period of exposure should have extended aspirin's effectiveness and this did not occur. Regardless of when aspirin treatment was begun in the present study, its antihypertensive effect was not evident when the rats reached 100–120 days of age. We must assume that it is the age of the rat which is critical to the antihypertensive action of aspirin and not an adaptation of some enzyme system or process over time.

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