SHORT COMMUNICATION

SUPPRESSION OF PLASMA RENIN BY ATEROGENIC LEVELS OF SERUM CHOLESTEROL IN RABBITS

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SUMMARY

1. Sixteen rabbits received either a normal or a high cholesterol diet over an 80-day period.
2. Diastolic blood pressure remained unchanged in each group.
3. Progressive suppression of plasma renin activity and concentration without change in renin substrate occurred in animals receiving the high cholesterol diet.
4. Kinetic studies excluded the mediation of enzyme inhibitors in this response and made probable a true suppression of renin secretion.
5. Commonly recognized influences seemed not to account for the findings and the possibility is raised of a dependence of renin secretion upon serum cholesterol. The effect occurs at the level of serum cholesterol found in man.

Key words: renin secretion, blood pressure, risk factors in hypertension.

Elevated serum cholesterol and high blood pressure are two widely accepted risk factors predisposing to heart attack and stroke (Pickering, 1972). Recently, a correlation has emerged between plasma renin activity (PRA) and predisposition to heart attack and stroke in essential hypertension (Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard & Buhler, 1972); in this work it was concluded that high PRA should itself be viewed as a risk factor and that suppressed PRA when it occurs may be a 'physiologic treatment for hypertension better than any medicine can devise'.

The present work is offered not as a study planned deliberately to test this concept but more as findings with apparent relevance to this important observation.

METHODS

Eight male New Zealand white rabbits were fed a standard chow of 'Barastoc' growers pellets and were given tap water ad libitum. The pellets contained 0.3% NaCl and provided a sodium

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intake of approximately 2 mmol kg\(^{-1}\) day\(^{-1}\) at the average food intake of 125 g/day. A further eight were fed a diet containing 1 g of cholesterol and 3 ml of peanut oil (McMillan, Klatzo & Duff, 1954) added to 100 g of the same pellets with the same sodium content. This atherogenic diet was commenced after a control period of 3 weeks on the standard diet.

Diastolic blood pressure was measured twice weekly from the dilated central ear artery by direct needle puncture recording through a low displacement transducer (Statham P23dB) tracing on to an Offner Dynograph. Validation of this method for diastolic pressure measurement is given elsewhere (Campbell, Skinner & Day, 1973). Blood samples (2.5-3.0 ml) for plasma urea, PRA and plasma renin substrate (PRS) determinations were collected from a marginal ear vein into cooled centrifuge tubes containing 30 units of heparin. The plasma was separated and stored at \(-15^\circ\)C. One blood sample was collected weekly during the 3 week control period and further samples were taken at 15, 50 and 80 days thereafter. Blood for cholesterol estimation was collected in similar manner but without anticoagulant. The serum thus obtained was stored at 4°C and analysed by the method of Zlatkis & Zak (1969) within 1 week of collection.

PRA, a measure of the reaction rate between endogenous renin and endogenous substrate at pH 7.5, was determined on 0.5-1.0 ml of plasma by the method of Skinner (1967) adapted to the rabbit (Campbell et al., 1973). PRS was measured as angiotensin released by angiotensinase free rabbit or human renal renin at a concentration sufficient to drive the reaction to completion in 10 min at 37°C without further change over 120 min. Plasma renin concentration (PRC) was calculated from the PRA and PRS of each sample by using the Michaelis–Menten equation and a calculated \(K_m\) of 2.3 \(\times\) \(10^{-6}\) m. Validation of this approach to renin assay is described elsewhere (Campbell et al., 1973).

The possibility that hypercholesterolaemic plasma might contain substances that interfere in the reaction between renin and its substrate was tested by adding low concentrations of rabbit renin to normal and hypercholesterolaemic plasma and comparing the increment in reaction velocity.

**RESULTS**

In the normal-fed animals all measured variables remained constant during the 80-day period (Fig. 1). Cholesterol feeding produced a progressive hypercholesterolaemia, rising from 0.26 mg/ml in normal-fed rabbits to 14 mg/ml after 80 days on the diet. Whereas cholesterol feeding was without effect on diastolic blood pressure, PRS or urea, there was a progressive suppression of PRA and PRC. This can be seen in Fig. 1 as increasingly significant differences between the values in cholesterol-fed and control animals over the 80-day period. Within the cholesterol group the slopes of the regressions of PRA and PRC on either serum cholesterol or time were significantly different from zero (PRA on cholesterol \(y = 8.34 - 0.254x\), \(P < 0.001\); PRC on cholesterol \(y = 44.4 - 1.26x\), \(P < 0.02\); PRA on time \(y = 8.28 - 0.052x\), \(P < 0.001\); PRC on time \(y = 43.5 - 0.24x\), \(P < 0.05\); \(n = 22\)). For the control group, regressions were not different from zero. In addition, when comparing the mean levels of PRA and PRC for the cholesterol-fed group with their own prediet level there was a significant suppression of each at 50 days and beyond (\(P < 0.01\), Student’s ‘t’-test for unpaired data). The levels of PRA and PRC in the control animals did not change over the same period.

To test if suppression of plasma renin was due to the presence of enzyme inhibitor in hyper-
Serum cholesterol on plasma renin

cholesterolaemic plasma, rabbit renin at twice the normal plasma concentration was added to both normal and hypercholesterolaemic plasmas. The detailed results are given in *Clinical Science and Molecular Medicine* Table 73/20 (deposited with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, from whom copies may be obtained). After correction for both the angiotensin formation owing to endogenous renin (PRA) and differences in substrate concentration ($V_{\text{max}}$) reaction rates in each type of plasma were of similar magnitude.

![Graph showing effect of high cholesterol intake on diastolic blood pressure (BP), plasma renin activity (PRA), substrate (PRS) and concentration (PRC) and on plasma urea and serum cholesterol.](image)

**Fig. 1.** Effect of high cholesterol intake on diastolic blood pressure (BP), plasma renin activity (PRA), substrate (PRS) and concentration (PRC) and on plasma urea and serum cholesterol. ⋄, Normal-fed (n=8); ○, cholesterol-fed (n=8). Values expressed as mean ± SE. Significance levels (*) relate to the difference between the two groups at particular times by using Student's 't' test for unpaired data: *P<0.1; **P<0.02; ***P<0.002; ****P<0.001. The differences in renin levels between normal and cholesterol-fed animals in the control period are not significant at the 10% level. Within the cholesterol group, the regressions of PRA and PRC on either serum, cholesterol or time are significant (Results). No pre-cholesterol diet values of serum cholesterol are shown since (see the text) they are too small for clear indication on the scale used.
The further possibility was considered that suppression of renin levels was due to factors other than the cholesterol intake. Control animals displayed a significant weight gain from $3.114 \pm 0.149$ g to $3.659 \pm 0.093$ g over the 80-day period (means $\pm$ SE, $n=8$, $P<0.01$, Students' 't'-test for unpaired data). Cholesterol-fed animals did not gain weight over the period ($3.011 \pm 0.177$ g to $3.202 \pm 0.130$ g, $n=8$, n.s.). By the end, but not at the beginning of the experiment, control animals were significantly heavier than the cholesterol-fed group ($P<0.02$). Food and water intake was not deliberately documented but it was noted that intake of solids was decreased in some of the cholesterol-fed animals late in the 80-day period. The usual water intake of 20–25 ml/day was not noticeably affected.

When examined at 100 days, moderate atherosclerosis of grade III–IV within a O–V scale (Day & Wilkinson, 1956) was present in all those animals fed on cholesterol.

**DISCUSSION**

Suppression of plasma renin by an atherogenic diet makes unlikely the possibility that renin is a contributing factor in the pathogenesis of this type of experimental vascular disease. Nor did elevation of diastolic pressure contribute to the disease. Diastolic pressure has not been previously measured in experimental atherosclerosis although elevation in systolic pressure has been reported by Bronte-Stewart & Heptinstall (1954) but not substantiated by either Shapiro & Seecof (1925) or Whittington-Coleman & Carrier (1970).

The suppression of plasma renin with an atherogenic diet was due to a genuine decrease in renin concentration since the assay methods excluded the influence of substrate concentration. Further, the kinetic studies showed the absence of cofactor influence. Suppression is then most likely due to a decrease in secretion although an increase in the metabolic clearance rate of renin must also be considered. The notion of increased clearance rate causally and permanently suppressing renin levels seems most unlikely, however, since such an event could only lead to a commensurate increase in renin secretion.

Although it is tempting to implicate elevated serum cholesterol as the cause of suppressed renin secretion in these experiments the relationship may not be one of direct dependence. Although recognizable influences such as increased renal perfusion pressure and altered cation intake can be reasonably excluded, other influences such as a change in adrenal steroid metabolism cannot be denied. In this respect, Albrecht, Kahnt, Neher & Schuler (1965) reported suppression of corticosterone secretion with cholesterol feeding in rabbits without a fall in blood concentration or protein binding, suggesting a decrease in metabolic clearance rate of corticosterone.

The question must remain open, but the most interesting possibility is that serum cholesterol alters renal handling of sodium, causing a small increase in extracellular fluid volume with compensatory suppression of renin secretion.

The final serum cholesterol level reached with the present diet is about 50-fold that for normal rabbits but only 5-fold higher than for normal man. Since renin suppression was progressive, the possibility might reasonably be entertained that the serum cholesterol level of atheroma-prone Western man is a controlling factor in renin secretion, a possibility made more acceptable by the fact that plasma renin activity of normal man ($1.7$ ng ml$^{-1}$ h$^{-1}$; Skinner, 1967) is considerably less than that of the rabbit ($10$ ng ml$^{-1}$ h$^{-1}$) on a comparable sodium intake.
Heart attacks and strokes are, in the main, the result of atherosclerotic vascular disease, with serum cholesterol an established risk factor (Pickering, 1972). If the dependence of plasma renin upon cholesterol of the type suggested by the present results were also true for man, it would discount the likelihood of a simple relationship between low plasma renin levels and protection from heart attack and stroke in essential hypertension.

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