Rapid effects of parathyroid hormone(1–34) and prostaglandin E₂ on bone blood flow and strontium clearance in the rat in vivo

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ABSTRACT

The vascular effects of noradrenaline, ATP, parathyroid hormone (PTH) and prostaglandin E₂ (PGE₂) were investigated in the rat. Additionally, the exchange of mineral ions between bone and blood was assessed by measuring strontium clearance, with the aim of investigating whether the vascular effects of these agents altered uptake of mineral ions or if this exchange could be changed independently of blood flow. Radioactive microspheres and ⁸⁵Sr were used to establish bone blood flow and mineral clearance. Measurements of bone blood flow and arterial pressure were made in each animal and used to calculate vascular resistance. A measurement of ⁸⁵Sr clearance was also obtained. Arterial blood pressure was significantly affected by noradrenaline (P ≤ 0.003) and ATP (P ≤ 0.015). Additionally, noradrenaline significantly (P ≤ 0.03) reduced bone blood flow. This decrease was related to a significant increase in vascular resistance. Arterial blood pressure and bone blood flow were significantly reduced by both bovine PTH(1–34) (P ≤ 0.001, P ≤ 0.02) and PGE₂ (P ≤ 0.005, P ≤ 0.001). Vascular resistance to bone was increased by both agents but this was only statistically significant in the case of PGE₂ (P ≤ 0.01). A significant (P ≤ 0.01) reduction in strontium was also produced by PGE₂. In each group the relationship between bone blood flow and strontium clearance was then analysed. Only the PGE₂-treated group had a slope of the regression which was statistically different from both the control animals and the other drug-treated groups. Treatment with PGE₂ therefore resulted in a dose-related decrease in ⁸⁵Sr clearance which was not related to the reduction in bone blood flow. Journal of Endocrinology (1991) 131, 359–365

INTRODUCTION

Previous work in this laboratory using the perfused canine tibia has shown that injection of metabolic inhibitors directly into the tibia nutrient artery significantly increased the net extraction of ⁸⁵Sr (McCarthy & Hughes, 1986). The results indicate that the metabolic activity of bone actively controls the efflux of ions from the exchangeable mineral pool and therefore the distribution of ⁸⁵Sr. Recently it has been demonstrated that parathyroid hormone (PTH) and prostaglandin E₂ (PGE₂) produce a rapid inhibition of ⁴⁴Ca uptake in chick and rat bone (Shaw & Dacke, 1985; Dacke & Shaw, 1987). These authors suggested that this represented a novel inhibitory mechanism which could be reconciled with the plasma calcium model of Talmage & Grubb (1977) but conceded that a vascular mechanism could be involved. These results conflict with those of Parsons & Robinson (1971), who found an initial influx of Ca into bone. It has been suggested that this influx was an artefact due to post-mortem migration of radionuclides into bone (Dacke & Shaw, 1987).

It has already been demonstrated that PTH and PGE₂ have hypotensive actions in many vascular beds (Charbon, 1968; Messina, Weiner & Kaley, 1976; Pang, Janssen & Yee, 1980a; Pang, Yang, Phillips & Yee, 1980b), vasodilatation of blood vessels resulting in reduced blood pressure. Using an isolated dog tibial perfusion model, Driessens & Vanhoutte (1981) investigated the effect of PTH. It was apparent that in the perfused situation the bone vascular bed shows no
response, with the lack of effect on induced constriction suggesting that no vasodilatatory action occurs. Little work, however, has been done to determine the vascular effects of those substances in bone, and therefore only inferences can be drawn as to their action.

In the present study we have investigated the effect of bovine (b)PTH(1–34) and PGE₂ on clearance of ⁸⁵Sr by long bones of the rat. Simultaneously, blood flow was measured using radioactively labelled microspheres, so that vascular effects associated with PTH and PGE₂ administration could be investigated. The relationship between blood flow and clearance is complex. Therefore, in order to separate vascular and non-vascular effects of PTH and PGE₂ on bone, comparison was made with blood flow and clearance changes produced by noradrenaline and ATP. Thus, this study was designed to investigate the influence of vascular factors on ⁸⁵Sr clearance in bone, and whether PTH and PGE₂ inhibited mineral uptake through vascular effects, or by other means.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 300–350 g were anaesthetized using sodium pentobarbital (0·1 ml/100 g) injected i.p. After dissection, a cannula was inserted via the carotid artery into the left ventricle, and a second cannula inserted into the caudal artery. All animals were connected to a Harvard physiological recorder for measurement of mean systemic blood pressure and heart rate. Measurements of blood flows were made using microspheres labelled with ⁵⁷Co and ¹¹³Sn (NEN research Product, Du Pont (U.K.) Ltd, Stevenage, Herts, U.K.) and mineral exchange was assessed using ⁸⁵Sr (Amersham International plc, Amersham, Bucks, U.K.). The use of microspheres with different labels allowed two measurements of blood flow to be obtained, so that animals could act as their own controls. ⁸⁵Sr was used as a calcium tracer because it is a γ emitter, and its radioactivity may be separated easily from that of ⁵⁷Co and ¹¹³Sn.

In two groups of animals the vasoactive agents noradrenaline and ATP (Sigma Chemical Co., Poole, Dorset, U.K.) were administered to examine the effects of changing blood flow on strontium clearance. These results were then compared with the effects of known calcium-regulating agents bPTH(1–34) and PGE₂ (Sigma Chemical Co.). A range of concentrations was selected from doses used by other workers; PTH and PGE₂ concentrations were taken from Dacke & Shaw (1987) and noradrenaline and ATP concentrations from Davies (1986). Each animal acted as its own control, i.e. the post-treatment value was compared with the pretreatment value (control) which was set as the baseline value.

In each animal a control value of blood flow was established using ⁵⁷Co microspheres (approximately 200 000, 5 μCi, 15 μm diameter) injected via the carotid cannula while withdrawing blood from the caudal cannula at a rate of 0·2 ml/min.

Either bPTH(1–34) or PGE₂ was then injected or noradrenaline or ATP was infused. Withdrawal of blood from the caudal artery was begun 30 s after injection and 30 s later the ¹¹³Sn microspheres (200 000, 5 μCi) and ⁸⁵Sr (0·5 μCi) were injected into the carotid cannula. Blood was collected for a total of 4·5 min. Infusion was over 5 min, via the carotid cannula (6 ml/h). The following doses were infused 0·25, 0·75, 1·25 mg ATP/animal and 1·0, 1·5, 2·0 μg noradrenaline/animal. Both PTH and PGE₂ were injected in a vehicle of 2 ml Krebs buffer via the carotid cannula (PTH, biological potency of 70%; 4, 8, 12 μg/animal; PGE₂; 20, 40, 60, 80 μg/animal, initial dilution of 1000 μg in 0·5 ml ethanol which acted as the carrier). Additionally, in some animals (zero groups) an equal volume of Krebs Ringer buffer was administered to give values which were used as the control for comparison of the ⁸⁵Sr measurements. There were a minimum of four animals in a dose group.

In the experimental design, each group of six animals obtained were given differing doses (including none). This was to reduce the effect of differences between groups of animals, which had been observed in previous studies.

At the end of the experiments the animals were killed using KCl injected via the tail cannula. Both tibiae and femora were quickly removed to minimize post-mortem migration of mineral ions into bone (Tothill & MacPherson, 1978). All soft tissue was carefully removed from the bones. The blood samples and bones were then analysed individually for radioactivity (Wallac γ counter). The values for each bone were then summed to give a total for each animal. In each case, the number of microspheres in the collected bone or summed bone samples was greater than 1000.

Bone blood flow and ⁸⁵Sr clearance were calculated using the following equations:

\[
\text{Bone blood flow} = \left( \frac{\text{[microsphere activity in bone]}}{\text{[microsphere activity in blood]}} \right) \times \text{blood withdrawal rate}
\]

\[
\text{⁸⁵Sr clearance} = \left( \frac{\text{[⁸⁵Sr activity in bone]}}{\text{[⁸⁵Sr activity in blood]}} \right) \times \text{blood withdrawal rate}
\]

The values were then divided by the weight of bone to give the results in the form of ml/min per g.

Vascular resistance was estimated from arterial blood pressure and blood flow by the following equation:

\[
\text{Vascular resistance} = \frac{\text{mean arterial pressure}}{\text{blood flow}}
\]

To analyse the dose–response, linear regressions were fitted to the data points. A form of the t-test was then used to see if the slopes of regression were statistically different from each other or from 0.

\[
t = \frac{b_1 - b_2}{\sqrt{\text{var} b_1 - b_2}} \quad \text{on } (n_1 \text{ and } n_2)
\]

Where \( b \) = the slope of the line of regression and the standard error of the slope is the square root of its variance.

RESULTS

Control

The initial mean control values are those measured during the \(^{57}\text{Co}\) estimation, i.e. pretreatment. For the 72 animals the mean blood pressure was 1.0 ± 0.02 Pa, the mean bone blood flow 0.3 ± 0.014 ml/min per g and mean vascular resistance 3.5 ± 0.2 Pa/min per g.

Strontium clearance was only measured once, thus the mean clearance values are for those animals that

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FIGURE 1. Linear regression for the dose–response for noradrenaline (□) and ATP (■) on (a) arterial blood pressure, (b) bone blood flow, (c) vascular resistance and (d) strontium clearance in the rat. Control animals received Krebs buffer only. Data are shown as means ± S.E.M. (n = 4 or 5 for each point.)
received the vehicle only (0.22 ± 0.02 ml/min per g, n = 17).

Effects of noradrenaline and ATP

Figure 1a shows the change in arterial blood pressure in response to noradrenaline and ATP. As expected, both these agents had a significant effect on systemic pressure. Arterial pressure was increased by noradrenaline (P ≤ 0.003) and decreased by ATP (P ≤ 0.015).

Both agents affected bone blood flow (Fig. 1b). Administration of noradrenaline caused a significant (P ≤ 0.03) dose-dependent decrease when compared with the pretreated values (P ≤ 0.03). Surprisingly, ATP also reduced blood flow. This, however, did not reach statistical significance because of the degree of scattering in the data (P ≤ 0.09).

Interpretation of the above findings is easier after calculation of changes in vascular resistance from the arterial pressure and blood flow (Fig. 1c). A significant (P ≤ 0.015) increase in resistance occurred in response to noradrenaline; at the highest dose the resistance was approximately double that of the 57Co control value. With ATP treatment there was a slight decrease in vascular resistance but this was not significant. It therefore seems that, unlike noradrenaline, there is no direct effect of ATP on bone, and that the changes in blood flow are a consequence of changes in arterial blood pressure.

The lines of regression for 85Sr clearance are plotted in Fig. 1d. In neither case was there any significant change in clearance as a consequence of the treatment; however, the noradrenaline group did show a slight, but statistically non-significant, decrease in 85Sr clearance (P ≤ 0.2).

Neither the noradrenaline- nor the ATP-treated animals (i.e. excluding control (0) animals from their respective groups) generated a line of regression that was significantly different from the control or from one another. It was therefore acceptable to combine these data to give one ‘vasoactive group’ as seen in Fig. 2. This group produces a line of regression which is significantly different from zero, indicating a correlation between flow and clearance (r = 0.7 slope of regression line (b) = 0.54, standard error of (b) = 0.11, n = 27).

Effects of PTH and PGE2

Both PTH (P ≤ 0.001) and PGE2 (P ≤ 0.003) produced statistically significant decreases in blood pressure (Fig. 3a).

Bone blood flow also showed a significant change in response to PTH and PGE2 treatment (Fig. 3b). The PTH group demonstrated a dose-dependent decrease in blood flow (P ≤ 0.02), with flow reduced by 57% at 12 µg. PGE2 also demonstrated this trend, and at the highest dose flow was reduced by 47% (P ≤ 0.001).

The change in vascular resistance in response to PTH and PGE2 is shown in Fig. 3c. Although both treatments resulted in an increase in this variable, this was only statistically significant in the case of PGE2 (P ≤ 0.01).

Figure 3d is a plot of 45Sr clearance for both PTH- and PGE2-treated rats. Once again both agents produced the same trend, that is a decrease in clearance with increasing dose. This trend, however, reached statistical significance (P ≤ 0.001) only in the case of the PGE2-treated group. In fact, in this study, this is the only agent that produced a significant effect on the clearance of 45Sr. It should also be noted that the overall decrease in bone blood flow seen in the PGE2-treated group was not greater than in the other groups.

The clearance of 45Sr is plotted against bone flow for both PTH and PGE2 in Fig. 4. The PTH-treated animals give a good correlation (r = 0.83) with a slope of regression of 0.5 compared with the value of 0.54 for the ‘vasoactive group’ and 0.51 for the control group. The PGE2-treated animals also displayed this relationship. Again there was a good correlation (r = 0.77) but the slope of the regression was 0.78, compared with 0.51 for the control group and this was a significant (P ≤ 0.01) difference. This increase in the slope for the regression line suggested that PGE2 decreased 45Sr...
clearance by a greater amount than could be ascribed to changes in flow.

**DISCUSSION**

The amount of $^{85}$Sr taken up by bone depends on the rate of blood flow; the permeability of the capillaries in the bone; the extravascular fluid space volume; the uptake rate into bone from the fluid space; and the back diffusion from the fluid space into the vascular system. Clearance measurements reflect the proportion of tracer delivered by blood to bone that is retained by bone over a specified time period. Thus clearance measurements are preferable to uptake measurements when there are significant vascular effects to consider.

However, the relationship between blood flow and clearance is not simple (McCarthy, Hughes & Orr, 1980), and therefore it was felt necessary in the present work to compare the effects of PTH and PGE$_2$ with the vascular effects produced by noradrenaline and ATP.

The vascular reactivity of bone has been described previously (Driessens & Vanhoutte, 1979; Gross, Heistad & Marcus, 1979), and the observations of noradrenaline increasing vascular resistance in bone are in agreement with these papers. Nutton, Fitzgerald, Brown & Kelly (1984) infused ATP into the right femoral artery of dogs and found that blood flow to the right tibia was increased when compared with the left. Based on these findings, ATP was expected to increase the blood flow to bone. Our experiments did not,
Theoretically, permeability blood Vanhoutte PTH produced exhibits resistance. However, as expected, hypotensive effects were demonstrated by both PTH and PGE$_2$. Neither agent, however, produced a significant decrease in bone vascular resistance. In fact, there was an increase in vascular resistance which was significant for PGE$_2$. In the case of PTH this is consistent with the findings of Driessens & Vanhoutte (1981) who showed that this hormone is not vasoactive in the isolated perfused tibia model.

Noradrenaline produced a significant decrease in blood flow resulting in an associated decrease in $^{85}$Sr clearance, although this was not significant. Only if $^{85}$Sr clearance were limited by blood flow would there be a direct proportionality between blood flow and $^{85}$Sr clearance. In the case of diffusion limitation, capillary permeability limits clearance (McCarty et al. 1980). Theoretically, an asymptotic relationship would be expected, with clearance approaching a constant value with increasing flow. In practice, as shown by Figs 2 and 4 relating blood flow and $^{85}$Sr clearance, it is often possible to plot a linear relationship between the two, with the constant of proportionality being less than one. Given the changes in blood flow observed and the slope of the regression line relating blood flow and clearance, the magnitude of the clearance changes are what would be expected. It is possible that our method does not produce precise enough measurements to show significant changes in clearance.

Administration of PGE$_2$ resulted in a statistically significant reduction in $^{85}$Sr clearance. A significant difference was also shown in blood flow by the PGE$_2$-treated group. In addition, a significant difference was also found in the regression line relating blood flow and clearance between the control and PGE$_2$-treated groups. In the control group it appeared that clearance was directly related to flow. This significant difference exhibited by the PGE$_2$-treated animals suggested that another factor was acting in this case, in addition to the vascular effect, i.e. the change observed in clearance was not purely mediated through changes in blood flow. This view supports the observation of Shaw & Dacke (1985), that the inhibition of calcium uptake is by some novel mechanism. The effects are, however, small when compared with the vascular effects. Our observations do not confirm that PTH acts in the same manner on bone, apart from its affect on bone blood flow. However, the effects observed by Dacke & Shaw (1987) in rat bone were less than seen in chick bone. Given the significant vascular effects on bone of systemic injection of PTH and PGE$_2$, it would seem preferable that experiments intended to examine the effects of PTH and PGE$_2$ on mineral exchange between blood and bone use a perfused organ technique (McCarty & Hughes, 1986).

The differences between the present results and those reported by Dacke & Shaw (1987) could be an age-related effect. We used rats of 300–350 g, whereas they used rats of 70–100 g when 3–4 weeks old, and 80–100 g chicks when 10–12 days old. The bone turnover will be much lower in the older animals and responses much reduced. The larger animals used in this study were required so that the arteries were sufficiently large to cannulate.

Anaesthesia could be another source of difference. Although all animals in the present study received the same anaesthetic treatment, it has been shown that anaesthesia for 1 h reduced bone blood flow in rabbits by 24% (Davies, Holloway & Pooley, 1990). The interval between blood flow estimations in the present study was typically 5 min, but the cannulation procedure could take up to 30 min. It is possible that the anaesthesia could modify responses relevant to normal homoeostasis.

In summary, PGE$_2$ significantly decreases both bone blood flow and $^{85}$Sr clearance, and increases the
vascular resistance of bone. The reduction in clearance appears to be independent of the reduction in blood flow. These results can be reconciled with the model of Talmage & Grubb (1977) in which calcium enters bone by passive diffusion, a calcium gradient between bone fluid and systemic extracellular fluid being maintained by a 'metabolic pump' located in surface osteocytes.

Finally this paper illustrates the problems of using the clearance technique to examine bone blood flow. Previous work has shown that extraction is dependent upon flow, and this work shows that extraction does not necessarily remain constant. Therefore, care must be taken when interpreting data in terms of blood flow.

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