# Haematological influences of potassium adaptation in normotensive and renally-hypertensive Wistar rats

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*Accepted: 20 March 2002*

**ABSTRACT**

Dietary potassium is known to cause reduction in blood pressure in several models of hypertension in human and animal studies but its haematological effects are not known. Here, experiments are designed to study the haematological effects of potassium adaptation (achieved by administering 0.75% KCl solution in drinking water for five weeks) in Wistar rats. The animals are divided into four groups comprising controls, potassium-adapted, renal hypertensive, and renal hypertensive with later adaptation to potassium. Packed cell volume (PCV) and platelet count (PC), whole blood and plasma viscosities, and platelet aggregation in the presence of sodium nitroprusside, levcromakalim, and glibenclamide, are studied. Results showed comparable PCV and PC in all groups. While relative whole blood viscosity was significantly higher ($P<0.05$) in the hypertensive group, relative plasma viscosity was similar in all groups. Adaptation significantly reduced ($P<0.05$) the tendency of platelets to aggregate to collagen. Sodium nitroprusside significantly reduced ($P<0.05$) the pro-aggregatory effects of collagen only in the control group. Neither of the potassium-channel modulators (levcromakalim, glibenclamide) caused any significant alteration in platelet response to collagen at the concentrations studied. Although these results suggest that potassium adaptation may not affect haemorheology, the reduced ability of platelets to aggregate – by mechanisms not clearly understood – has implications for reduced thromboembolism and the attendant cardiovascular sequelae.

KEY WORDS: Nitroprusside. Platelet aggregation. Potassium. Potassium channels.

## Introduction

Chronic oral potassium intake above that ordinarily available in foods (adaptation) causes reduction in blood pressure in several models of hypertension in the rat,[1-5] and human studies support this. Unpublished data shows a similar phenomenon in the normotensive rat. Some reports,[6] however, indicate no change in arterial pressure after potassium supplementation, although the protocols seem to vary with the groups of workers.

Mechanisms suggested to account for these findings include increased endothelium-mediated relaxation[5,7] and increased sodium/potassium ATPase activity[8] in the aorta of rats given potassium-supplemented diets.

Involvement of the endothelium might suggest a role for an endothelium-derived relaxing factor, possibly nitric oxide. Platelets are known to synthesise nitric oxide, which has anti-aggregatory properties in these cells.[9-11] They contain $SKC_a$, intermediate $KC_a$, and $K_v$ potassium channels, and the presence of $K_{ATP}$ has not been excluded completely.[12]

A reduced tendency for platelets to aggregate could result in decreased blood viscosity and a reduction in the incidence of intravascular blood clotting.[13] However, no direct link has been found between haematological function and potassium supplementation, or alteration in platelet response following the administration of potassium-channel modulators. Furthermore, it is not known if potassium supplementation could cause a change in platelet response to pro-aggregatory agents.

Here, we study the haematological influence of potassium administered as potassium chloride in drinking water on blood obtained from rats, in order to clarify some of the current uncertainty.

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## Materials and methods

***Animals***

Adult male Wistar rats weighing 220.3 ± 17.08 and 227.1 ± 26.7g (mean ± standard deviation) for the control and other groups, respectively, were obtained from the Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. They were housed in standard cages, fed rat chow (Livestock Feeds plc, Nigeria), allowed access to a particular drinking fluid *ad libitum*, and exposed to a 12-h light-dark cycle. The animals were allowed to acclimatise to these conditions for two weeks, and then were divided into four groups comprising controls, potassium-adapted (adapted), renal hypertensive (RHP), and renal hypertensive with later potassium adaptation (RHP-A).

**Fig. 1.** The effect of potassium adaptation on relative whole blood (RWBV) and plasma (RPV) viscosity (*$P<0.05$). The numbers between the bars represent the number of animals from which blood was obtained.

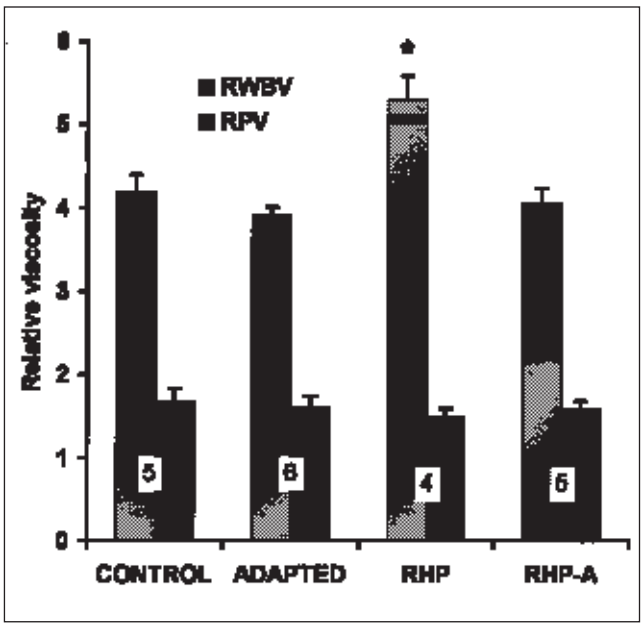
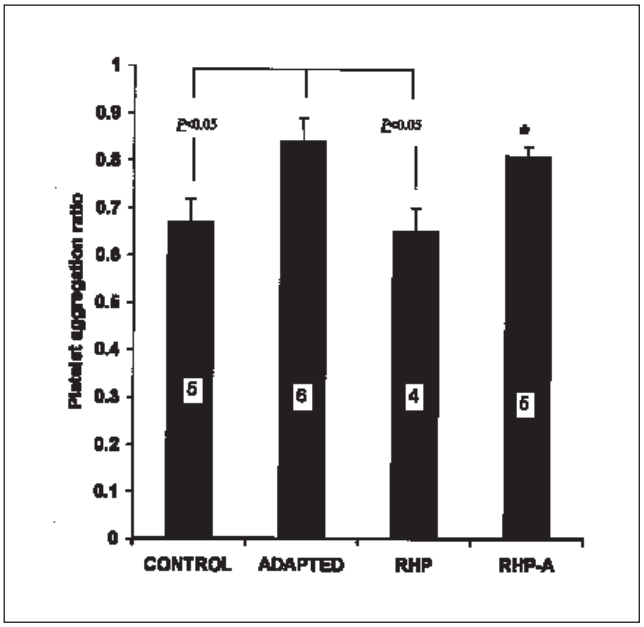



**Fig. 2.** The effect of potassium adaptation on collagen-induced platelet aggregation (*$P<0.05$). The numbers in the bars represent the number of rats from which blood was drawn.




Potassium adaptation was achieved by giving the animals 0.75% potassium chloride for five weeks. Renal hypertension was induced by the method first described by Grollman[14] and used by Eferakeya and Osunkwo.[15] Under pentobarbitone anaesthesia (40mg/mL), a unilateral nephrotomy was performed and the contralateral kidney compressed in a figure-of-eight pattern by a ligature. Thereafter, these rats were given 0.9% sodium chloride solution in place of tap water for six weeks. In the RHP-A group, 0.75% potassium chloride replaced normal saline for five weeks after hypertension was established.

*Measurement of blood pressure*

Mean arterial pressure (MAP) was measured directly in rats anaesthetised with pentobarbitone (40 mg/kg [ip]) by connecting a physiological pressure transducer (Bentley Trandec, USA) to a heparinised cannula inserted into the carotid artery. Animal body temperature was maintained at 36 ± 2°C by means of an overhead lamp, around which a thermometer was fitted, and respiration was facilitated by tracheal intubation. Recordings were taken using a two-channel Gemini 7070 recorder (Ugo Basile, Italy).

*Blood sample collection and haematological experiments*

Rats were anaesthetised as previously described and blood samples were collected via a carotid cannula into citrate or EDTA (for aggregation studies) bottles. Samples were maintained at 22°C for 15 min and quantities of blood were withdrawn for the particular experiments. Packed cell volume (PCV) and platelet count (PC) were estimated in platelet-rich plasma under phase-contrast microscope by the method of Dacie and Lewis.[16] Relative whole blood viscosity (RWBV) and plasma viscosity (PV) were measured by the method of Reid and Ugwu,[17] described in detail by Famodu *et al*.[18]

**Table 1.** Mean arterial pressure (MAP), packed cell volume (PCV) and platelet count (PC) by group ($n=5$ in each group)

| | MAP (mmHg) | PCV (%) | PC (x103/μL) |
|---|---|---|---|
| Control | 110.8 ± 2.8 | 32.0 ± 1.5 | 257.2 ± 25.6 |
| Adapted | 95.6 ± 5.0* | 33.2 ± 2.1 | 242.3 ± 19.0 |
| RHP | 138.2 ± 4.1 | 35.5 ± 1.7 | 262.5 ± 30.0 |
| RHP-A | 116.0 ± 4.4** | 35.8 ± 3.0 | 259.0 ± 13.9 |

*$P<0.01$
**$P<0.05$

*Platelet aggregation studies*

Platelet aggregation was monitored using the method of Wu and Hoak.[19] Briefly, platelet aggregation was examined in blood samples anticoagulated either with EDTA plus formalin solution or EDTA plus phosphate buffer, and an aggregation ratio was calculated. Blood (0.5 mL) was withdrawn into two separate EDTA tubes, drugs were added and then 2.5 mL of either formalin solution or buffer were added. After thorough mixing, the tubes were stored at 22°C for 15 min and then centrifuged at 150 x*g* for 8 min. Platelet counts were performed on the two samples.

Platelet aggregation was studied in this way in the presence of i) 150 μg (60 μL of 2.5 mg/mL) collagen alone as an aggregating agent; ii) 2.5 μmol/L (12.5 μL of $10^{-4}$M) sodium nitroprusside (SNP), followed 10 min later by 150 μg collagen; iii) 2.5 μmol/L (12.5 μL of $10^{-4}$M) levcromakalim (LEV), followed 10 min later by 150 μg collagen; and iv) 3 μmol/L (15 μL of $10^{-4}$M) glibenclamide (GLIB), followed 10 min later by 150 μg collagen.

Subsequently, platelet aggregation ratios were calculated for each of the samples. The doses of drugs (except collagen) were based on the concentrations from our unpublished

data that produced 70% relaxation (sodium nitroprusside and levcromakalim), or inhibition of relaxation (glibenclamide) in isolated rat aorta.

*Drugs*
Sodium nitroprusside (Sigma, UK) was prepared fresh for each experiment. Pentobarbitone sodium (Sigma) was prepared weekly by dissolving in distilled water. Glibenclamide and levcromakalim (Smith Kline Beecham, UK) were freshly prepared in absolute and 70% ethanol, respectively, with further dilutions carried out in 10% ethanol-water mixture. Collagen (Behring Werke Ag Marburg, Germany) was dissolved in distilled water.

*Statistical analyses*
In all cases, data are presented as the mean ± standard error of the mean (SEM). Comparisons were made where appropriate by ANOVA with Tukey *post hoc* test (GraphPad Prism Software). $P<0.05$ was regarded as significant.

## Results

*Effects on packed cell volume and platelet count*
Table 1 shows the MAP, PCV and PC for the groups studied. The protocol significantly reduced MAP in both normotensive and hypertensive rats. No differences were seen in PCV within the groups, suggesting that the protocol had no effect on the parameter.

*Effects on blood and plasma viscosity*
The results are show in Figure 1. The RHP group showed a higher RWBV ($P<0.05$), with a value of 5.30 ± 0.29. RPV did not change significantly. RHP and RHP-A groups gave values of 1.50 ± 0.09 and 1.58 ± 0.1, indicating that blood and plasma rheological was not affected by adaptation.

*Effect on platelet aggregation*
Adaptation appeared to decrease the pro-aggregatory effect of collagen (Figure 2). Platelets from potassium-adapted rats showed significantly higher aggregation ratios (0.84 ± 0.05, $P < 0.05$) than did the control (0.67 ± 0.05) and the RHP groups (0.65 ± 0.02). The RHP-A group showed values similar to those in the adapted group (0.81 ± 0.02), indicating facilitated antiplatelet activity due to later exposure to potassium.

*Effect of drugs on platelet aggregation*
Addition of 150 µg collagen to blood containing 2.5 µmol/L SNP did not produce a significant change in platelet aggregation (Figure 3), except in the control group (from 0.67 ± 0.06 to 0.83 ± 0.04, $P<0.05$). Overall, SNP appeared to reduce the tendency of platelets to aggregate, but this did not achieve significance. Values for collagen and SNP followed by collagen were 0.84 ± 0.05 and 0.89 ± 0.01 (adapted), 0.65 ± 0.05 and 0.80 ± 0.06 (RHP), and 0.81 ± 0.02 and 0.84 ± 0.02 (RHP-A), respectively.

Figure 4 shows the platelet response to collagen-induced aggregation in the presence of levcromakalim. A pattern similar to that obtained with SNP was seen; however, this did not achieve significance. Platelet aggregation ratios for the groups were 0.67 ± 0.06 and 0.80 ± 0.04 (control), 0.84 ± 0.05 and 0.88 ± 0.02 (adapted), 0.65 ± 0.05 and 0.75 ± 0. 0.03 (RHP), and 0.81 ± 0.02 and 0.86 ± 0.03 (RHP-A).

**Fig. 3.** Effect of 150 µg collagen on blood samples pretreated with 2.5 µmol/L SNP (*$P<0.05$).

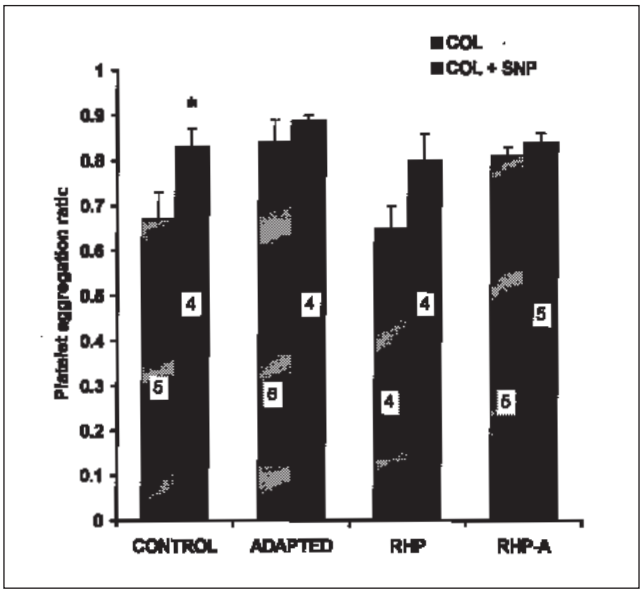
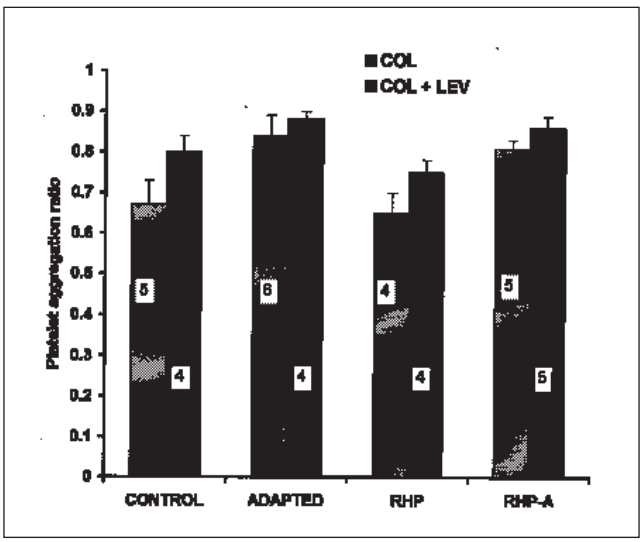



**Fig. 4.** Effect of pretreatment with 2.5 µmol/L levcromakalim on collagen-induced platelet aggregation.

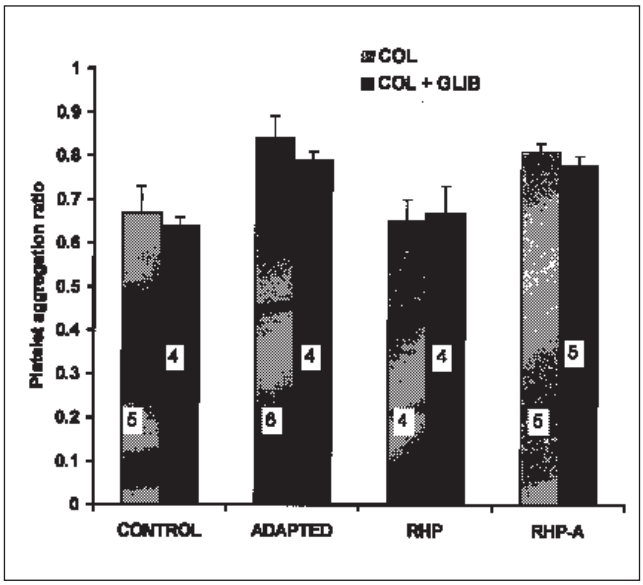



Glibenclamide appeared to enhance collagen-induced platelet aggregation (Figure 5), but not significantly. Mean aggregate ratios were lower in all but the RHP group as follows: 0.67 ± 0.06 and 0.64 ± 0.02 (control), 0.84 ± 0.05 and 0.79 ± 0.02 (adapted), 0.65 ± 0.05 and 0.67 ± 0.06 (RHP), and 0.81 ± 0.02 and 0.78 ± 0.02 (RHP-A).

## Discussion

Generally, diet provides the nutrients required for the synthesis of the formed elements of blood, haemoglobin and other plasma proteins. The roles of iron in haemoglobin formation and of calcium as a coagulation factor are well known, but less is known of the role of potassium. Thus, while the BP-lowering effect of potassium adaptation is

**Fig. 5.** Effect of pretreatment with 3 μmol/L glibenclamide on collagen-induced platelet aggregation.




supported by the results of this study, the short- and long-term haematological consequences remain unknown.

In the present study, potassium adaptation did not affect PCV or PC. A reduction in platelet count in patients with pre-eclampsia has been reported[20] but whether or not this occurs in other hypertensive situations is not known. Taken together, the absence of any obvious change among the groups studied here provides a basis for comparison of the effect of drugs.

Hypertension has been reported to increase the rheological properties of blood.[21,22] This is consistent with the current findings but the mechanisms underlying the increased viscosity of blood has yet to be fully explained. Increased fibrinogen levels and concomitant derangement of fibrinolytic activity occur in hypertension and these may account, at least in part, for this increase.[22,23] However, other workers[24] have reported that blood viscosity and fibrinogen concentration decrease in hypertension. Increased viscosity leads to further elevation in BP, due to increased stasis and peripheral resistance. Plasma viscosities in the other groups were also similar, implying that arterial resistance, particularly at capillary level, may be responsible for the increased viscosity in the RHP group.

Although there are many methods available to study platelet aggregation, the method used in the present study was a manual one. Although regarded as a drawback, the method is useful (if extreme care is taken) in situations where facilities for more widely used methods are lacking.

Platelets in potassium-adapted and RHP-A groups showed a lower propensity to aggregate, and implies that potassium adaptation is responsible. Whether or not adaptation activates ATPase in the platelets is not known.

The effect of SNP on platelet activation has been reported previously.[11,25,26] Nitric oxide donating agents are known to possess antiplatelet activity, and the mechanisms are diverse and the subject of intensive research. So far, facilitation of the phosphorylation of thromboxane receptor by cGMP-dependent protein kinase,[27] decreased expression of P-selectin,[28,29] fibrinogen binding *in vitro* and *in vivo*,[28] and inhibition of thrombin receptor-activating peptide-induced phosphoinositide-3-kinase activity[30] in human platelets have all been suggested.

In the present study, SNP reduced the tendency of platelets to aggregate and this is consistent with the findings of previous reports.[11,26] The finding that SNP did not reduce collagen-induced aggregation significantly in the potassium-adapted group may be due to the fact that little original aggregation was seen in this group. In the other groups the concentrations of SNP and collagen might be important.

This may also hold true for the influence of levcromakalim pretreatment on collagen-induced platelet aggregation, where results indicated a non-significant increase in platelet aggregation ratios. Levcromakalim would be expected to open potassium channels in platelets; however, previous studies have not suggested any possible consequence of this. Perhaps potassium-channel opening leads to hyperpolarisation and a reduction in the ability of the contractile apparatus (in the dense tubules) to initiate the necessary platelet response.

If potassium-channel opening inhibits platelet aggregation, closure of the channels might be pro-aggregatory, and glibenclamide would be expected to enhance collagen-induced aggregation. However, reductions in platelet aggregation ratio were not significant in the present study. No report is available on the influence of potassium-channel blockade on platelet function.

Impaired $KC_a$ channel function is seen in platelets from patients with Alzheimer's disease[31] but the consequence of this on platelet function is unknown. Once again, the concentration of the blocker might be critical to the response.

In conclusion, although these results suggest that potassium adaptation may not affect haemorheology, the reduced ability of platelets to aggregate – by mechanisms not clearly understood – has implications for reduced thromboembolism and the attendant cardiovascular sequelae. In addition, potassium-channel modulating drugs may, at high enough concentration, alter the response of platelets to aggregating agents. □

*The work was supported by a University of Benin URPC grant to RIO. The authors are grateful to the staff of the Department of Pharmacology & Toxicology, University of Benin, for their support.*

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