The Mechanism of the Vasodilator Action of Potassium[1] (36560)

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An elevation of potassium concentration over the range 4 to 12 mEq/liter in the blood perfusing a vascular bed produces a decrease in vascular resistance (1–4) most likely due to relaxation of vascular smooth muscle. Reduction of the plasma potassium concentration to about one-half normal or less produces an increase in resistance (5–7) attributable to smooth muscle contraction (8). Thus, over the approximate range of 0 to 12 mEq/liter, potassium is a dilator. The mechanism of the dilation is unknown. One possibility is that the changes in external potassium concentration affect the activity of the Na^+–K^+ activated ATPase in the membrane of the vascular smooth muscle cell. We have therefore examined this hypothesis by observing the resistance response of the collateral-free canine gracilis muscle to hyperkalemic and hypokalemic perfusion before and after the administration of ouabain, a well-known inhibitor of the Na^+–K^+ sensitive ATPase activity (9, 10).

Materials and Methods. Dogs of either sex weighing 20–25 kg were anesthetized with sodium pentobarbital (30 mg/kg, intravenously). The right gracilis muscle was surgically isolated except for the gracilis artery and vein. Both ends of the muscle were tied to exclude collateral flow. The animal was then heparinized (5 mg/kg). Arterial blood from the left femoral artery was pumped at a constant rate into a hemodialyzer and then to the gracilis artery; flow rate was initially adjusted such that gracilis perfusion pressure approximately equaled systemic arterial pressure (7). Gracilis perfusion and systemic arterial pressures were continuously monitored. Arterial blood was sampled before and after the dialyzer for the determiniation of plasma potassium concentration, osmolality and hematocrit.

In the control period the blood was dialyzed against a modified Ringer solution (7). Hypokalemia was produced by dialyzing the blood against the modified Ringer solution containing no potassium. Hyperkalemia was produced by dialyzing against the modified Ringer solution containing twice normal potassium (8.4 mEq/liter). The dialysate osmolalities were maintained isosmotic by altering the amount of NaCl in the solution. The gracilis muscle was challenged with hypokalemic and hyperkalemic blood both before and after close intra-arterial infusion of a solution of ouabain (10 μg/ml at the rate of 0.25 ml/min for 20–40 min). In some experiments the resistance response to faradic stimulation of gracilis nerve (6 cps, 1.6 msec, 6 V) and close intra-arterial injection of norepinephrine, acetylcholine or adenosine was tested before, during and after ouabain administration. The data were analyzed with Student's t test modified for pair duplicates.

Results. The experimental sequence as well as a representative tracing is shown in Fig. 1. Before ouabain, hypokalemia (from 3.9 to 1.4 mEq/liter) produced a rise of 15 mm Hg in perfusion pressure; hyperkalemia (from 3.9 to 6.0 mEq/liter) produced a decrease of

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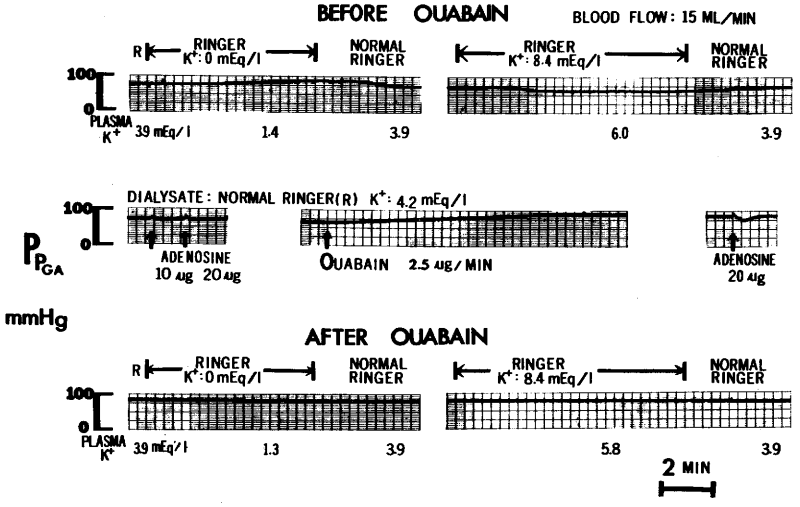

FIG. 1. Tracing from a representative experiment. P_{PGA} represents gracilis perfusion pressure. Plasma [K^+] is that in arterial blood perfusing the gracilis.

13 mm Hg. Ouabain itself (2.5 µg/min) produced a gradual rise in perfusion pressure which reached a maximum by the eighth minute of ouabain infusion. After ouabain, neither hypokalemia (from 3.9 to 1.3 mEq/liter) nor hyperkalemia (from 3.9 to 5.8 mEq/liter) produced a change in perfusion pressure. The preparation still responded to adenosine injection. Average data from 12 such experiments are shown in Figs. 2–4.

Figure 2 shows that ouabain was without effect on systemic arterial pressure. Perfusion pressure gradually rose and attained a maximum value in an average of 8 min. It then gradually fell (ouabain infusion continuing) reaching a steady state in an average of 20 min. The latter pressure level was not different from that in the control period.

Figure 3 shows that ouabain completely inhibited the vascular response to hypokalemia. Indeed, in 4 of 12 animals the response to hypokalemia was actually reversed when compared to both the pre- and postcontrol values.

Figure 4 shows that the fall in perfusion pressure produced by hyperkalemia was greatly attenuated after ouabain. In 5 of 12 animals the fall in perfusion pressure produced by hyperkalemia was converted to a rise after ouabain.

Plasma osmolality and hematocrit were not altered by the dialyzer and were constant throughout the experiment. In these experiments as well as those described below, the vasculature still responded normally to norepinephrine, acetylcholine and adenosine.

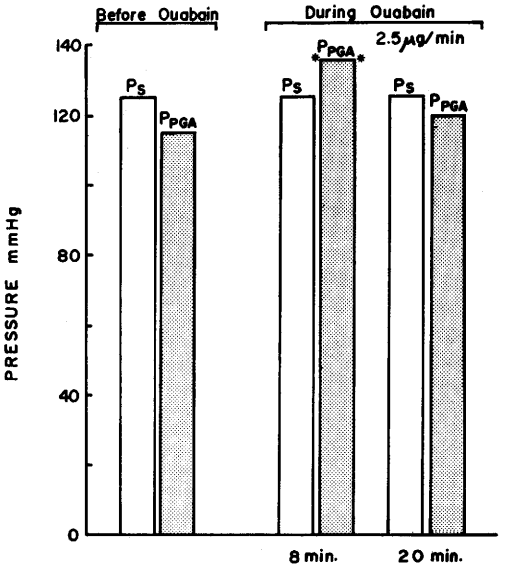

FIG. 2. Average effects of ouabain on gracilis perfusion pressure (P_{PGA}) and systemic arterial pressure (P_S) at minutes 8 and 20 of infusion ($n = 12$). Ouabain was given intra-arterially into the gracilis artery. * denotes a significant change at $p < .05$ level relative to the control or minute 20 of ouabain infusion.

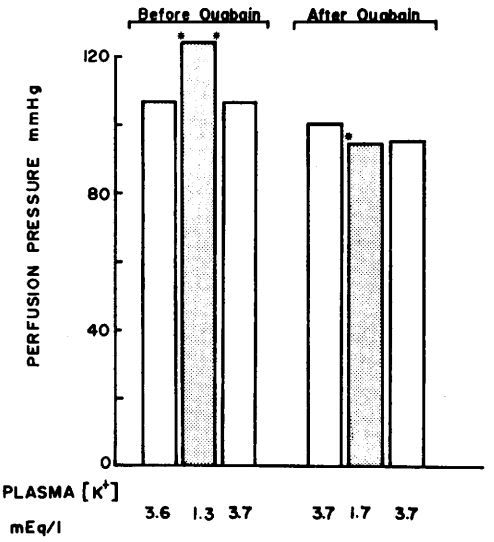

FIG. 3. Average effects of hypokalemia on gracilis perfusion pressure before and after intra-arterial ouabain infusion (2.5 μg/min) ($N = 12$). Average gracilis blood flow was 15.8 ml/min. * denotes a significant difference at $p < .05$ level from precontrol (* shown on the left corner) or from postcontrol value (* shown on the right corner).

Similar experiments were performed on four isolated canine forelimbs also at constant inflow. The preparation used permitted separation of skin and muscle outflow (11). Hypokalemia increased resistance proportionally in skin and muscle while hyperkalemia decreased resistance proportionally in both vascular beds. Ouabain blocked the response to hypokalemia and greatly attenuated the response to hyperkalemia.

In five animals gracilis nerve stimulation before administration of ouabain produced the usual response, *i.e.*, an abrupt marked fall in vascular resistance. After ouabain the magnitude of the resistance fall produced by nerve stimulation was only slightly attenuated, even though the response to hyperkalemia was greatly diminished. Two characteristics of the response were, however, regularly affected; these were the onset of the vascular response to nerve stimulation and the time required for perfusion pressure to reach a steady-state value during stimulation. Before ouabain nerve stimulation produced an immediate (within 1–2 sec) fall in perfusion pressure and pressure reached a minimum stable value in less than 10 sec. After ouabain the onset of the response was delayed to approximately 5 sec and the time required for perfusion pressure to reach a minimum stable level was prolonged to approximately 20 sec. Identical results were obtained from three additional experiments (two gracilis muscles and one forelimb) in which the dialyzer was omitted from the extracorporal perfusion circuit. In these preparations, ouabain blocked the vasodilator response to hyperkalemia (5.5 to 11.1 mEq/liter, produced by infusion of isotonic potassium chloride). A sharp rise in resistance, indicating potassium contracture, still occurred when potassium concentration was raised to high level.

Discussion. This study shows that, in the anesthetized dog, (a) the response of the skin and skeletal muscle vasculatures to local administration of ouabain is biphasic, *i.e.*, resistance initially rises then gradually declines to a level that is the same or slightly below the pre-ouabain level; (b) the response of these circulations to hyperkalemia and hypokalemia is drastically modified by ouabain (that to hyperkalemia is greatly attenuated, completely blocked or reversed and that to hypokalemia is either blocked or reversed); (c) the response to simulated ex-

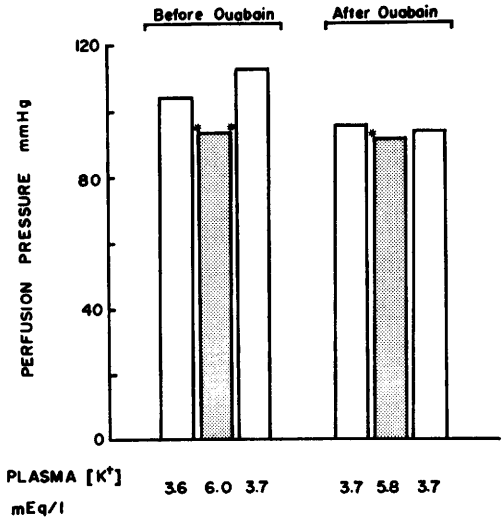

FIG. 4. Average effects of hyperkalemia on gracilis perfusion pressure before and after intra-arterial ouabain infusion (2.5 μg/min) ($N = 12$). Blood flow and * as in Fig. 3.

ercise, though still present, is altered in time course after ouabain. These vascular alterations occur at a time when the responses are normal to several other vasoactive agents, indicating that the effect of ouabain is not entirely nonspecific.

Analysis of these *in vivo* data in view of *in vitro* studies of others leads to the following hypothesis regarding the mechanism of the vasodilation of potassium. It is proposed that deviation in the plasma potassium concentration either slightly above or below normal elicites a change in the activity of the Na^+–K^+ ATPase (Na^+–K^+ pump) located in the membrane of the vascular smooth muscle cell. Since there is *in vitro* evidence that the Na^+–K^+ pump is electrogenic (pumps more Na^+ out than K^+ in), these changes in activity result in cellular loss or accumulation of positive charges opposite to those predicted from the passive ion fluxes. Consequently, cell potential changes are also opposite from those predicted. It is well established that a change in cell potential is normally associated with a change in the contractile state of vascular smooth mucle, *i.e.*, hyperpolarization is associated with relaxation and hypopolarization with contraction. According to the hypothesis, a slight increase in plasma K^+ (4 to 12 mEq/liter) increases the activity of the Na^+–K^+ pump resulting in a net loss of positive charges from the cell. The loss of positive charges (hyperpolarization) leads to smooth muscle relaxation. The reverse occurs when plasma potassium is reduced below normal.

Results obtained from *in vitro* studies support this hypothesis. When the bathing medium surrounding certain isolated tissues is changed from one having a normal potassium (4 mEq/liter) to one having a slight excess of potassium (4–12 mEq/liter), the cells hyperpolarize rather than depolarize (12). On the other hand, when potassium is removed from the medium depolarization is observed (12, 13). These changes in cell potential cannot be explained by passive ion fluxes (Nernst equation) nor an electroneutral pump (Goldman equation) and hence, appear to be related to an electrogenic pump. Studies have demonstrated that the activity of extracted cell membrane Na^+–K^+ ATPase is enhanced in the presence of high potassium and retarded in the presence of low potassium (14). Moreover, it is known that this membrane Na^+–K^+ activated ATPase is involved in the active transport of Na^+ and K^+ across the cell membrane (9, 10).

These *in vitro* studies are compatible with the hypothesis that the *in vivo* vasodilation of potassium is the result of a change in cell potential mediated through a stimulating effect of potassium on an electrogenic Na^+–K^+ pump. The most compelling *in vivo* evidence in support of the hypothesis, however, is that ouabain, a Na^+–K^+ ATPase inhibitor, alters the resistance response to potassium. Following ouabain the vasoconstrictor response to low potassium is blocked or reversed and the vasodilator response to high potassium is attenuated, blocked or reversed. These postouabain effects of potassium are now more in accord with those expected from passive ion fluxes (Nernst and Goldman equations). The initial rise in skin and skeletal muscle vascular resistance produced by ouabain is also compatible with the hypothesis, assuming ouabain inhibits the cell membrane Na^+–K^+ pump in an *in vivo* preparation. Stopping or reducing the rate of an electrogenic pump should cause intracellular accumulation of positive charges, depolarization and contraction. Furthermore, the rise in resistance produced by ouabain or low potassium and the fall in resistance produced by high potassium all support the existence of a Na^+–K^+ ATPase sensitive electrogenic pump *in vivo*. The secondary fall in resistance that always occurred during and following infusion of ouabain, however, is not predicted and is unexplained.

The effect of ouabain on exercise dilation is noteworthy since potassium has been assigned an important role in this response by several investigators (2, 6, 15). The present studies detract from this possibility because the overall response to exercise is not markedly altered in a vascular bed rendered essentially unresponsive to potassium. However, following ouabain both the onset of exercise dilation and the time to reach maximum dilation were delayed. It is possible

that potassium or some other ouabain-sensitive vasodilator normally initiates the dilation and is then reinforced by other ouabain-insensitive vasodilators such as adenosine, adenine nucleotides, osmolality, hydrogen ion, etc. (16). Further investigation is necessary to clarify this question.

Summary. In the dog gracilis muscle and forelimb, ouabain blocked or reversed hypokalemic constriction and suppressed, blocked or reversed hyperkalemic vasodilation. This suggests that the vasodilator action of potassium is related to stimulation of membrane Na^+–K^+ ATPase activity resulting in hyperpolarization and relaxation of the vascular smooth muscle cell. Exercise dilation was only slightly modified in a muscle in which potassium vasodilation was greatly suppressed, suggesting a minor role for potassium in sustained exercise hyperemia.

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