Effect of Potassium on the Resting Length of Vascular Smooth Muscle of the Rabbit Aorta and its Response to L-Norepinephrine

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In certain forms of experimental hypertension the concentration of potassium in the wall of the large arteries is increased. It is known that the properties of vascular smooth muscle are greatly altered by changes in extracellular potassium, and the possibility must be considered that potassium may be involved in the mechanism of experimental hypertension.

The experiments described below were designed to study the effects of changes in extracellular potassium concentration \([K^+]_o\) on the basal tone, the contractile response to L-norepinephrine and the tissue concentration of potassium \([K^+]_t\) of helical rabbit aorta strips. The results suggest that in this preparation when \([K^+]_o\) is increased, the change in resting length due to the potassium change is more important than the changes in the response to L-norepinephrine. The changes in sensitivity to L-norepinephrine may be related to the intracellular potassium concentration.

Methods

Helical rabbit aorta strips were used throughout this study. Four preparations from each animal were mounted as described previously in separate baths containing Krebs solution equilibrated with 95% O\(_2\) and 5% CO\(_2\). Isometric contractions were recorded with a lever magnification \(\times 20\) and a tension of 2 g. After an initial equilibrium period of two hours, the responses of the tissue to successive concentrations of L-norepinephrine, 0.02, 0.08, 0.2 \(\mu\)g/ml were recorded. From these results the maximum response, the median effective dose and the slope of the dose-response curve were determined. This provided an initial check of the sensitivity and relative contractility of the four tissue preparations in normal Krebs solution (K\(_+\), 5.9 mEq/l). Then, using one tissue as a control the potassium concentrations in the other baths were changed every hour in random sequence. Potassium concentrations of 50, 150, 200, 250 and 300% normal were employed. Forty minutes after each change of potassium, the change in base line (resting length) was measured and the characteristics of response to L-norepinephrine determined as before and compared to those of the control determined at the same time. Changes in resting length of the experimental strips are expressed as percentage of the maximum response which would have obtained had the strip remained in normal Krebs solution. This “expected” maximum response was determined from the response of the control strip after corrections for its relative sensitivity and for base line drift.

The osmotic pressure of the bathing solution was maintained by compensatory changes of sodium chloride or choline chloride. The results obtained with both substances were identical and will be treated together in results. Each observation was repeated on vessels from 12 different rabbits.

Rabbit aorta strips were analysed for their potassium content using the technique previously described. One of the four strips from each rabbit was placed in normal Krebs solution, the others in solutions with differing potassium concentrations. These concentrations were the same as those listed above. After two hours equilibration they were removed, wiped on a tile and then transferred to oven-dried hard glass test tubes for weighing. The loss of weight after overnight heating at 105°C gave the water content. The desiccated tissue was digested with 0.1 ml coned nitric acid and 0.1 ml water in a heated water bath for one hour; the contents were diluted with 3 ml water and reheated for a further hour. The contents were further diluted to 25 ml, for estimation on a flame photometer against external standards. Each measurement of total potassium was repeated on eight rabbits.

Tissue potassium, K\(_t\), was determined by sub-
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POTASSIUM CONCENTRATION AS % NORMAL

FIGURE 1

Effect of changes in concentration of potassium in Krebs bicarbonate solution on the resting length (A) and on the maximum contraction (B), median effective dose (C) and the slope of the dose-response curve (D) to l-norepinephrine of the helical rabbit aorta strip. Decrease in resting length is expressed as a percentage of the maximum response, and the other parameters expressed as a percentage of the corresponding value in normal Krebs bicarbonate solution. Limits represent SE of the mean of observations from a minimum of 12 animals.

Results

A. CHANGES IN RESTING LENGTH

Changes in resting length are expressed as a per cent change of the expected maximum response of the tissue to l-norepinephrine in normal Krebs solution.

In figure 1A the relationship between \([K]_o\) and the resting length is illustrated. An increase in \([K]_o\) shortened the resting length, i.e., increased the resting tone: a decrease in potassium had the opposite effect.

B. CHANGES IN THE CHARACTERISTICS OF RESPONSE TO L-NOREPINEPHRINE

Maximum Response

The maximum response to l-norepinephrine at different potassium concentrations expressed as a percentage of that expected in normal Krebs solution is illustrated in figure 1B. At all values of \([K]_o\) tested, the con-
The results described in the previous sections show that when [K]₀ is changed during exposure to a constant level of L-norepinephrine, the final degree of shortening depends on a number of factors. The resting length, the slope of the dose-response curve, and the sensitivity of the tissue to the drug all vary with the concentration of extracellular potassium. In figure 2 the relative importance of these factors in the change of length of an "average" aorta strip is illustrated using the mean values of the results obtained in all these experiments. The increased shortening associated with the addition of potassium is predominantly due to a change in resting length: the decreased shortening following a reduction in potassium is primarily due to a decreased sensitivity to L-norepinephrine. The tendency toward a change in maximum response dampens the effect of increased L-norepinephrine sensitivity seen at higher potassium concentrations and potentiates the effect of decreased amine sensitivity at lower potassium concentrations.

C. RELATIONSHIP BETWEEN INTRACELLULAR POTASSIUM AND SENSITIVITY TO L-NOREPINEPHRINE

By combining the results described above with those in which the potassium content of arterial strips was measured, it was possible to obtain corresponding values of tissue potassium, Kᵯ, extracellular potassium, K₀, and median effective dose of L-norepinephrine. Possible interrelationships were sought between these values, and it was found that the MED was a function of 1/[K]ᵯ, 1/[K]₀, and [K]₀/[K]ᵯ.

As no one function represented the data better than another, other information is required to decide which, if any, of these functions represents a causal relationship. If it is assumed that potassium exerts its contractile effect, not through cell membrane depolarization but by some other means, perhaps by acting on an intracellular site (see Discussion), then the first relationship between MED and 1/[K]ᵯ is the most likely to be
of causal significance, although the reasons for choosing this are somewhat empirical and inadequate. This relationship is illustrated in figure 3.

Discussion

These studies demonstrate that when the concentration of potassium bathing aortic vascular muscle exposed to L-norepinephrine is altered, the resultant change in length depends upon several factors. When \([K]_o\) is increased, change in resting length is more important than change in sensitivity to L-norepinephrine. When \([K]_i\) is decreased, the relative importance of these two factors is reversed. Bohr et al.\(^4\) described the increase in response of aortic strips to a constant submaximal dose of racemic epinephrine following addition of potassium, but did not consider the change in base line to be important. Recently, Barr et al.\(^8\) studying strips of stored dog carotid artery stated that the magnitude of the late tonic contraction seen when these strips recovered in various solutions at 38°C was related to \([K]_o\). The amplitude of the response to electrical stimulation was related to the \([K]_i: [K]_o\) ratio and \([K]_i\). Rondell and Gross,\(^6\) recording isometrically the contractions of various rabbit vessels, found that an increase in potassium increased the tension developed to submaximal doses of racemic norepinephrine. Although they state that no change in resting tension took place, their published record reveals that a small change did in fact occur.

When \([K]_o\) is increased, the rate of contraction of arterial muscle is similar to the rate of accumulation of intracellular potassium.\(^9,10\) In comparison, the rate of vascular muscle depolarization is about four times as fast as contraction (Su and Bevan, unpublished results). In view of these observations we consider it more likely that contraction is linked with the accumulation of intracellular potassium rather than the potassium induced membrane depolarization. As drug induced contractions may be initiated in the completely depolarized vascular muscle cell,\(^11\) a mechanism for contraction independent of membrane potential changes is present in this tissue. Such a mechanism may be affected by changes in \([K]_i\). There is also evidence that L-epinephrine acts within the cell membrane of normal aorta smooth muscle cells.\(^5\) It is because of these considerations that we favor the relationship between \(\text{MED}\) and \(1/([K]_i)\) as being of causal significance, although the basis for such a choice is not strong.

Such a conclusion concerning the intracellular site of action of K is similar in part to the conclusions of Barr et al.,\(^8\) who showed clearly that the shortening of recovering carotid strips was a positive function of intracellular potassium. However, if the tissues were first allowed to recover 6 mm \([K]_o\) and were then exposed to 12 to 24 mm \([K]_o\), the strips quickly shortened, presumably due to a change in the transmembrane K gradient, since an insufficient time for change in intracellular potassium had elapsed. We have never observed such a rapid change as this with the rabbit aorta strip. This may represent a species difference or else a difference between
arteries. The aorta does contain very primitive smooth muscle cells. Experimental hypertension is associated with changes in intracellular potassium of the large blood vessels; it is therefore relevant to discuss our results in relation to this experimental condition. Folkow states that in hypertension, changes in basal vascular tone, the result of local mechanisms, are more important than changes in the reactivity of the vascular muscle to liberated sympathetic transmitter. Tobian has emphasized that perhaps the most important difference between normotensive and hypertensive patients is the basal vascular resistance. Our experimental findings with aortic vascular muscle show that potassium increase causes a shortening due mainly to a direct action of the ion and only secondarily due to increased sensitivity to the sympathetic transmitter. If aorta muscle were characteristic of vascular muscle as a whole, these findings would suggest a causal role of potassium in experimental hypertension. However recent studies have shown that vascular muscle is not homogeneous. Quantitative as well as qualitative differences occur between vessels of different size and position. Indeed Overbeck has shown that some resistance vessels dilate rather than constrict with increase in [K+]. Consequently a general conclusion relating [K+] to hypertension is unwarranted on the basis of existing knowledge. However these studies do emphasize that changes in intracellular ion content affect not only the reactivity of vascular muscle to the physiological amines but also its basal tone. Such changes may be of importance in experimental hypertension.

Summary

Change in resting length, response to l-norepinephrine and tissue concentration of potassium of helical rabbit aorta strips have been investigated, following changes in concentration of extracellular potassium. Increase in potassium decreases resting length, increases sensitivity and slope of log dose — probit response curve: potassium decrease has opposite effects. Alterations in external potassium concentration have no effect on the maximum response to l-norepinephrine. A potassium range between 50% and 300% of normal was investigated. Results show that when the muscle shortened, following an increase in potassium in the presence of L-norepinephrine, the change in resting length due to a direct action of this ion was more important than changes in sensitivity to L-norepinephrine. It is suggested that sensitivity of muscle to L-norepinephrine may be related to the intracellular concentration of potassium.

References
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