5–6 μm, and 9–10 μm (Figures 1a, b). These diameters are smaller than those of cutaneous nerves supplying the calf of frogs[18].

A graph of the range of conduction velocities found in our experiments and those of CATTON[3] as a function of the range of peaks in the histogram of axon diameters shows that this relationship is linear (Figure 2). The conduction velocity values reported by CATTON are slightly higher than those reported here, but the linear relationship holds equally well for the conduction velocities found in this study. The present study demonstrates that the dorsal cutaneous nerve consists of relatively few axons of several distinct receptor classes. Since unitary action potentials are discernible in records from the whole nerve following physiological stimulation of the skin[9], it is possible to use this preparation in studies which analyze firing properties of populations of afferents.

Zusammenfassung. Der dorsale Hautnerv (rami cutanei dorsi mediales) des Frosches (*Rana pipiens*) enthält nur eine geringe Axonenzahl. Die Maxima im anatomischen Spektrum stimmen linear mit den entsprechenden Maxima im elektrophysiologischen Spektrum überein.

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Effect of Potassium and Norepinephrine on the Tone of the Isolated Artery: Changes by Ouabain Pretreatment

The vasodilating effect of an increase in the extracellular potassium concentration by 1–10 m*M* is well known from investigations on various vascular beds[1,2] as well as on isolated arteries[3]. The physiological significance of this potassium effect is indicated by the finding that the interstitial K^+ of skeletal muscle rises during muscular activity, and that the time course of the K^+ change is similar to that of the functional hyperemia[4,5]. Furthermore, an increase in K^+ in the vasodilating range inhibits the vasoconstriction in response to norepinephrine (NE) in the perfused dog forelimb[6] and in the isolated artery[7]. This effect is comparable to the 'functional sympatholysis' in working skeletal muscle[8]. The mechanism of both K^+ effects is not yet clear. It has been proposed that the dilating effect of K^+ might be related to changes in the membrane potential inverse to those of the K^+ diffusion potential and mediated by activation of the sodium pump[9]. Recently that assumption was supported by the finding that ouabain attenuates or prevents the vasodilating action of K^+ in the perfused dog gracilis muscle[10]. Therefore we investigated the influence of ouabain on the K^+ induced dilation and on the NE–K^+ interaction in arteries in vitro.

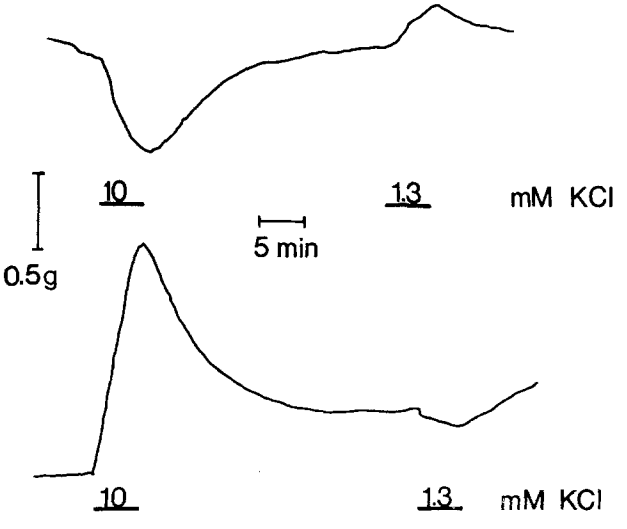

Fig. 1. Effect of changes in the KCl concentration of the rinsing solution (normal value 2.7 m*M*) on the tone of a helical strip from the bovine facial artery. Upper curve: normal response (basal tension 3 g). Lower curve: response after 120 min incubation with 5×10^{-8} g/ml ouabain (basal tension 4.2 g).

Methods. The experiments were performed on helical strips of bovine facial arteries. The arteries were stored overnight at 4 °C, cut, mounted in a moist chamber at 37 °C and rinsed continuously by physiological salt solution (in m*M* KCl 2.68, NaCl 136.88, $MgCl_2$ 0.49, $CaCl_2$ 1.36, $NaHCO_3$ 11.88, NaH_2PO_4 0.32, glucose 8.0). The strips were prestretched (basic tension 200–300 g/cm^2 or 3–5 g per strip, resp.), and equilibrated for 2 h prior to the experiments. The tension was recorded isometrically.

Results. When the potassium concentration of the rinsing solution was increased from 2.7 to 10 m*M*, the wellknown relaxation of the strips was observed. Reduction in the potassium concentration to 1.3 m*M* induced a constriction. This response pattern was changed after incubation of the arteries in ouabain containing physiological salt solutions (threshold about 2×10^{-8} g/ml ouabain). Ouabain itself (5×10^{-8} g/ml, 90 min incubation) increased the tone of the arterial strips by about 100%.

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In the ouabain pretreated artery, an increase in the KCl concentration to 10 mM led to a constriction, and a decrease to 1.3 mM to a relaxation. An example is shown in Figure 1. The effect of various ouabain concentrations on the reaction of the arterial strips to a change from 2.7 to 10 mM KCl is summarized in the Table.

When the normal rinsing solution was replaced for 5 min by a solution containing $1–5 \times 10^{-7}$ g/ml norepinephrine (NE), the strips contracted. This contraction was inhibited, when the rinsing solution contained 10 instead of 2.7 mM KCl (cf.[7]).

Incubation of the arterial strips in 5×10^{-8} g/ml ouabain reversibly enhanced the NE-induced constriction in 17 out of 18 experiments. This potentiation was more pronounced with 10^{-7} g/ml NE (increase by about 100%) than with 5×10^{-7} g/ml NE (increase by about 50%). After increase in the KCl concentration – resulting in a constriction of the ouabain-pretreated arteries- the increase in tension in response to NE was reduced in 14 out of 16 experiments (Figure 2). This effect was also reversible.

The absolute tension of the strips contracted by NE was about the same in 10 and in 2.7 mM KCl, because the inhibiting effect of 10 mM KCl on the NE-induced constriction was balanced by the constricting effect of 10 mM KCl on the ouabain-pretreated artery. When the rinsing solution was changed to isotonic KCl, the resulting constriction was much higher than that induced by 5 min NE application. Therefore the inhibition of the NE-induced contraction by 10 mM KCl cannot be attributed to a restricted contractility of the arteries.

The inhibiting effect of the increased K^+ -concentration on constricting agents is not restricted to NE.

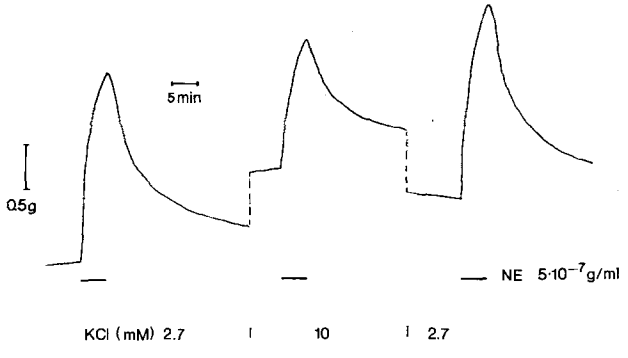

Fig. 2. Contraction of an arterial strip by NE in solutions containing 2.7 or 10 mM KCl and 5×10^{-8} g/ml ouabain. Pretreatment with ouabain for 90 min. When the KCl concentration was changed, the recorder was stopped for 40 min (dashed line).

Tension change of arterial strips in response to an increase in the KCl concentration of the rinsing solution from 2.7 to 10 mM after 90 min incubation in physiological salt solution containing various ouabain concentrations

Ouabain concentration (g/ml)	n	Tension change (mg) $\bar{x}$	$s\bar{x}$
0	39	– 237	36
1×10^{-9}	9	– 331	110
1×10^{-8}	4	– 200	67
5×10^{-8}	28	+ 840	118
1×10^{-7}	3	+1057	251

When arterial strips (with or without preincubation in 5×10^{-8} g/ml ouabain) were contracted by application of 1.5 mM $BaCl_2$ for 1–2 min (10 experiments), the same effect was observed. The Ba^{++}-induced contraction was not potentiated by ouabain.

Discussion. Ouabain in a concentration above 10^{-8} g/ml constricts the isolated arterial muscle, and changes the effect of a moderate increase in the outer K^+ concentration from vasodilation to vasoconstriction. These effects can be interpreted as an action on an electrogenic sodium pump. Inhibition of an electrogenic pump by ouabain or by reduction of the K^+ concentration in the bathing solution would cause a depolarization and a resulting vasoconstriction. Activation of the pump by a moderate increase in the outer K^+ concentration would have the inverse effect (hyperpolarization and vasodilation). When the pump is inhibited by pretreatment of the artery with ouabain, changes in the outer K^+ concentration only affect the K^+ diffusion potential. Now an increase in the K^+ concentration leads to a depolarization and vasoconstriction.

Our results are supported by similar findings obtained on the perfused dog gracilis muscle by CHEN et al.[10], and with regard to the vasoconstricting effect of ouabain by TREAT et al.[11].

Direct evidence for the participation of an electrogenic sodium pump in the generation of the membrane potential has been found in many tissues[12] including the smooth muscle[13, 14], but no precise data about the changes in membrane potential of arterial smooth muscle during variation of the extracellular K^+ concentration in the physiological range are available up to now. Therefore the final decision about the validity of the theory described above should be postponed until that gap is closed.

In our experiments the inhibiting effect of an increase in KCl from 2.7 to 10 mM on the vasoconstriction by NE or Ba^{++} persisted after incubation in ouabain, though the direct effect of the change in K^+ on the strip tension was inverted. This finding leads to the assumption that the extracellular K concentration might influence the arterial smooth muscle at different levels of the excitation-contraction coupling, and that its action might be too complex to be interpreted only as an action on an electrogenic sodium pump. The mechanism of the potentiating effect of ouabain on the NE-induced vasoconstriction cannot be explained on the basis of our results.

Zusammenfassung. Vorbehandlung von Arterienstreifen mit g-Strophantin ($> 10^{-8}$ g/ml) kehrt die mechanische Reaktion auf K^+-Änderungen im Bereich von 1 bis 10 mM KCl, nicht aber die K^+-Wirkung auf die Kontraktion durch Noradrenalin oder Barium um. Danach könnte die K^+-Dilatation der Arterien auf der Aktivierung einer elektrogenen Na^+-Pumpe beruhen.

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