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Effect of Ouabain on Skin and Skeletal Muscle Vascular Beds in the Dog Forelimb (40162)

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A local vasoconstrictor action of the cardiac glycosides is well established. They contract isolated vessels when added to the bathing solution (1–4) and raise the total resistance to blood flow through vascular beds when infused intraarterially (5–9). The vasoconstriction may not be sustained however. In a previous study in canine gracilis muscle (10), we observed that continuous intraarterial infusion of ouabain (2.5 μg/min) produced a progressive rise in resistance which reached a maximum in about eight minutes. Resistance then gradually waned, reaching the control level 20 min after starting the infusion.

We wondered whether waning of the vasoconstriction is peculiar to vessels in skeletal muscle and which types of vessels (large arteries, small vessels, large veins) are responsible for the constriction. We therefore prepared the dog forelimb in a manner which allows calculation of the total resistance to blood flow through both skeletal muscle and skin, as well as segmental resistances in series in each of these two vascular beds. We then infused ouabain into the brachial artery for one hour and compared the resistance responses in these two parallel vascular beds. We found that the responses are quite different.

Methods. Mongrel dogs of either sex weighing 17–23 kg were anesthetized with sodium pentobarbital (30 mg/kg IV) and ventilated with room air through an endotracheal tube with a Harvard positive pressure respirator.

The collateral-free, innervated forelimb preparation used in this study has been described in detail previously (11–13). In brief, after ligating the communicating branch between the cepahlic and brachial veins, brachial arterial inflow was held constant while measuring cephalic venous (skin) and brachial venous (muscle) outflow. Pressures were measured in the brachial artery and at three sites (small artery, small vein, large vein) along the length of each parallel (skin, skeletal muscle) bed. Arterial inflow was held constant with a blood pump, pressures were measured with a multiple transducer–oscillograph system, and venous outflows were measured using graduated cylinders and a stopwatch. A second pump was used to return blood to the animal via the femoral vein at the same rate it appeared. Total limb resistance, total skin resistance, total muscle resistance, and series resistances (large artery, small vessel, large vein) in skin and skeletal muscle were calculated from the appropriate flows and pressure gradients. Resistances are expressed in mm Hg/ml/min.

While the preparation does not provide complete anatomical separation of the skin and skeletal muscle vascular beds, separation is sufficiently complete to allow resistance calculations. Changes in total resistance in the two vascular beds in response to a variety of vasoactive agents are essentially those observed in a preparation where separation is antomically complete (12).

Three series of experiments were performed. In a first preliminary series of nine limbs, a solution of ouabain (Sigma Chemical Co., St. Louis, MO) in isotonic saline, 50 μg/ml, was infused into the brachial artery perfusion line upstream to the pump at 0.123 ml/min (6.4 μg/min) for 20 min. The infusion rate was then doubled (12.4 μg/min) for the next 20 min and then again doubled (24.7 μg/min) for a final 20 min. Since infusion at 6.4 μg/min produced significant changes, this infusion rate was explored for a more prolonged period of time in a second series of eight limbs; the drug was infused at this rate for the entire 60 min. Five additional animals served as a control series; nothing was infused. Pressures and flows were measured at 6.5-min intervals throughout the experiments.

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The data were analyzed using Student's *t* test modified for paired replicates. The values for each time period were compared to the preexperimental control value within the same group of animals. The level of significance was set at $P < 0.05$ as determined from a two-tailed table.

Results. Intrabrachial infusion of ouabain at 6.4 μg/min raised perfusion (brachial arterial) pressure from 136 ± 7 to 173 ± 6 mm Hg by the 20th min. It remained at this level until the 39th min and then fell to 161 ± 12 mm Hg by the 59th min. The pressures in the small skin and small muscle arteries responded similarly; they rose from control values of 109 ± 6 and 106 ± 6 mm Hg respectively to 140 ± 6 and 136 ± 8 mm Hg respectively at the 20th min, remained steady at these levels until the 39th min, and then fell to 127 ± 12 and 115 ± 13 mm Hg respectively at the 59th min. Cephalic (skin) and brachial (muscle) venous outflows remained at control levels for the first 20 min (Fig. 1). Cephalic venous outflow then gradually fell and brachial venous outflow gradually rose. Associated with the rise in brachial venous outflow was a small increase in muscle small vein pressure (from 10.3 ± 0.8 to 13.2 ± 1.9 mm Hg). Small skin vein (8.9 ± 0.8 mm Hg), cephalic (large skin) vein (5.4 ± 0.6 mm Hg), and brachial (large muscle) vein (7.6 ± 0.5 mm Hg) pressures did not change significantly throughout the experiment.

These findings suggest that initially both the skin and muscle vascular beds constricted at the small vessel level but that the constriction was not sustained in the muscle. The resistance calculations confirmed this impres-

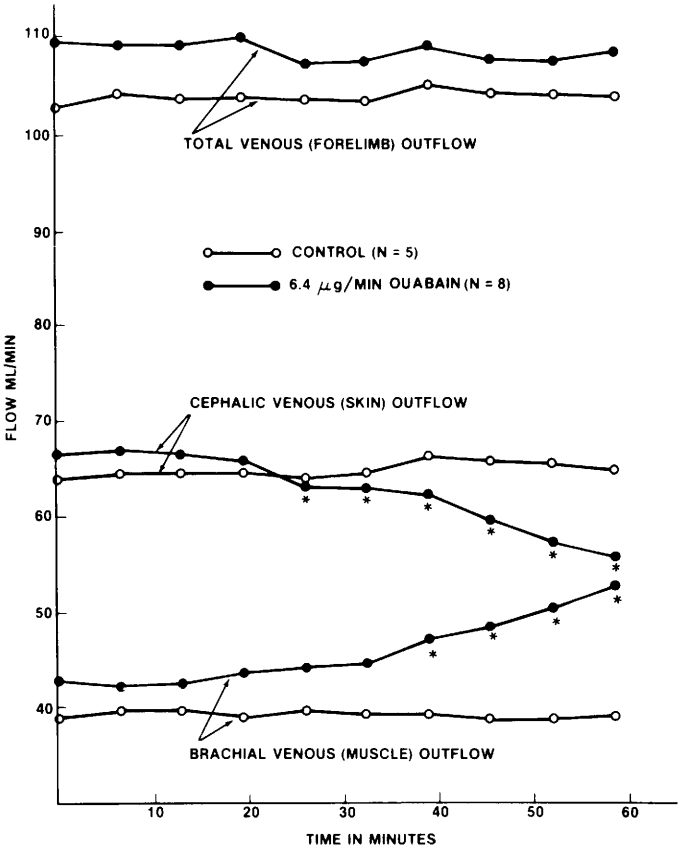

FIG. 1. Effect of ouabain on the outflow of blood from the brachial and cephalic veins of the dog forelimb with brachial arterial inflow held constant. The ouabain was infused intraarterially at 6.4 μg/min (50 μg/ml, 0.123 ml/min) for one hour starting at the second minute. Nothing was infused in the case of the control series. * = $P <$ 0.05 relative to value at zero time.

sion. Figure 2 shows that total skin resistance rose progressively due mainly to a rise in skin small vessel resistance; skin large artery resistance contributed slightly but skin large vein resistance not at all. On the other hand, total muscle resistance rose to a maximum by the 13th min and then progressively fell, reaching the control level by the 52nd min. The rise and fall in resistance resulted from changes in the muscle small vessel segment; muscle large artery resistance was slightly increased during the last half of the experiment (when total muscle resistance was falling) and muscle large vein resistance was unaffected. The net effect of all of these changes on total forelimb resistance was a gradual increase followed by a plateau and finally a waning of the increase.

These changes were not seen in the control series. Venous outflows remained constant throughout the experiment (Fig. 1). There were no statistically significant changes in resistance for the first 45 min. At the 52nd min skin and muscle small vessel resistances were slightly increased. The total forelimb, total skin and skin small vessel resistances were slightly elevated at the 58th min (a small spontaneous rise in resistance as a function of time is commonplace in this and other similar preparations, most likely due to a gradual reduction in blood volume subsequent to insensible water loss and slight heparin-induced bleeding). The resistance to flow through large arteries and large veins remained normal at all times.

Systemic arterial pressure did not change significantly in either series of experiments.

Discussion. When flow into an artery which supplies two parallel vascular beds is held constant, venous outflows from the two beds will reflect the resistance to flow through the two vascular beds (14). When the resistance in the two beds respond proportionately to a stimulus, venous outflows will not change. When they respond disproportionately, however, flow will shift from one vascular bed to

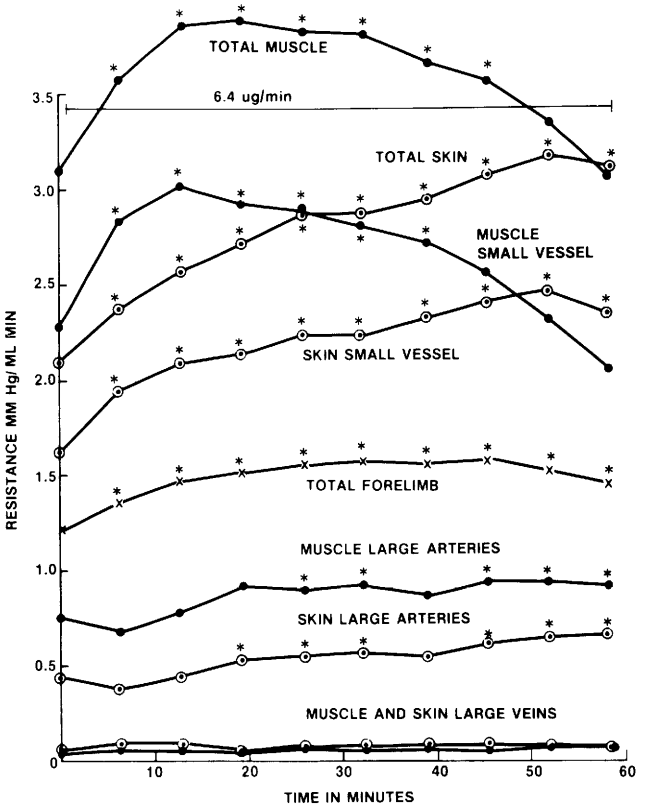

FIG. 2. Effect of ouabain infused into the brachial artery at 6.4 μg/min for one hour on parallel and series resistance in the dog forelimb perfused at constant flow. * = $P < 0.05$ relative to value at time zero. $n = 8$.

the other. During the first 20 min of the ouabain infusion, perfusion (brachial arterial) pressure rose but venous outflows did not change indicating that the skin and skeletal muscle vascular beds were constricting proportionately. Subsequently however, perfusion pressure failed to rise further and flow started shifting from skin to muscle indicating that constriction in muscle was no longer proportionate to that in skin. The flow shift progressed with time and, toward the end of the infusion, perfusion pressure fell back toward the control level. Calculations at this time indicated that the total resistance to flow through skin was greatly elevated whereas the total resistance to flow through muscle was now not different from control. They also showed that these changes in total skin and muscle resistance resulted mainly from resistance changes in the skin and muscle small vessel segments.

Thus these studies show that the forelimb skin and skeletal muscle vascular beds respond differently to ouabain. The constriction is sustained in skin but, as was observed previously in gracilis muscle (10), it wanes in skeletal muscle. The difference is found in the small vessel segment which contains the arteriole. Large arteries in the two beds respond similarly.

Ouabain is a well known inhibitor of the sarcolemmal Na,K-ATPase required for active Na^+ and K^+ transport. Since the transport is electrogenic in vascular smooth muscle (15, 16), ouabain, by blocking active extrusion of Na^+, could lead to depolarization, increased Ca^{2+} influx, increased intracellular free $[Ca^{2+}]$, and hence contraction of the smooth muscle cell. Alternatively or simultaneously, the increased intracellular $[Na^+]$ could lead to increased intracellular free $[Ca^{2+}]$ via the Na^+-Ca^{2+} exchange mechanism (17, 18). It is also possible that ouabain could constrict in part by influencing norepinephrine transport in the sympathetic nerve terminal and hence its concentration in the cleft between the terminal and smooth muscle cell (19).

The mechanism of the waning of the constriction in skeletal muscle has not been considered previously. Several possible mechanisms are 1) repolarization due to reactivation of the membrane Na^+, K-ATPase by the rise in intracellular $[Na^+]$, 2) uncoupling of the contractile elements from the membrane resulting in relaxation despite continued depolarization (as has been observed during prolonged potassium free perfusion of isolated arteries (15)), and 3) withdrawal of sympathetic nervous activity via central (20, 21) or carotid sinus baroreceptor (22, 23) actions of ouabain. However none of these possible mechanisms explain the absence of waning of small vessel resistance in skin.

It seems more likely that the waning is related to the type of tissue that surrounds the small vessels. Perhaps actions of ouabain on the skeletal muscle releases substances which dilate the arterioles. One such substance might be the potassium ion, which is known to be vasodilator (10). Other substances may also be involved since ouabain can increase the oxygen consumption of skeletal muscle (24). Release of vasodilators from skeletal muscle would also explain why large artery resistance was not affected similarily. The large arteries are located upstream to and thus not within range of the metabolites.

Summary. Ouabain was infused into the brachial artery of the dog forelimb for one hour and the resistances to blood flow through the skin and skeletal muscle vascular beds were calculated. Skin vascular resistance progressively increased whereas skeletal muscle resistance initially increased and then returned to the control value. This difference between the responses in these two parallel beds was entirely accounted for by changes in small vessel segments. There was no difference in the responses of the large arteries in the two beds.

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