Responses of Arterial Vessel Strips from Skin and Muscle to Serotonin[1] (35590)

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Serotonin is a unique vasoactive agent which may be vasopressor in some species but vasodepressor in others, and the pressor response in a given animal may be reversed by a change in basal conditions (1). Page (2) established that serotonin is depressor when pressure is neurogenically high and pressor when pressure is neurogenically low. In this regard, Haddy *et al.* (3) found that intra-arterial (ia) infusion of serotonin decreases total forelimb resistance when initial resistance is spontaneously high and increases limb resistance when initial resistance is neurogenically low. Similar observations have been in the dog hindlimb, renal and mesenteric vascular beds (4). Many studies have shown that serotonin is a potent vasoconstrictor of cutaneous blood vessels (5, 6). Studies from our laboratory also indicate that ia serotonin infusion always constricts cutaneous vessels in the dog forelimb (7, 8) and hindpaw (9) but may have little effect or dilate forelimb (7, 8) and gracilis (8, 9) muscle vessels. These observations have been confirmed by Abboud (10). Available evidence suggests that the variable effect of serotonin on muscle vessels may depend upon the initial muscle vascular resistance (9–11). The dilator action of serotonin in muscle has also been reported by Bock *et al.* (12) during ia or intravenous (iv) infusion or injection and by Takacs and Vajda (13) during iv infusion. The increase in skin resistance results largely from large artery and large vein constriction; whereas large arteries and large veins of muscle do not appear to respond (7).

The difference in the responses of *in situ* muscle and skin arteries larger than 0.5-mm diameter could be due to a difference in their direct response to serotonin or to some indirect action present in one but not the other, *i.e.*, release of a constrictor in skin or a dilator in muscle. The direct response of these larger arteries was therefore compared utilizing strips from metacarpal skin and gracilis muscle arteries.

Materials and Methods. Adult mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg) and anticoagulated with heparin sodium (3 mg/kg). The metacarpal and gracilis muscle arteries (1- to 1.5-mm diam) were surgically removed and immediately transferred to a physiological salt solution (PSS) maintained at approximately 37°. The composition of this PSS (mmoles/liter was: NaCl, 119; KCl, 4.7, KH_2PO_4, 1.18; $MgSO_4$, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; sucrose, 50; $CaCl_2$, 1.6. A fine wire was then threaded through the lumen of the vessel. By pressing the sharp edge of a scalpel blade against the wire at one end and directing the vessel into the blade, a helical strip was cut from the vessel as the wire was rotated. The strip, approximately 1 cm long, was mounted in a 90-ml plastic container filled with blood and maintained at 37°. One end of the strip was tied to a glass rod and the other end to a force transducer (Grass model FT.03). Strip tension was recorded with a Grass ink-writing oscillograph.

In order to have a physiological environment which approximated the *in vivo* situation, strips were bathed continually with circulating blood from a cannulated femoral artery of a donor dog. Inflow to the bath was maintained constant with a Sigmamotor pump and outflow of the bath was returned by gravity to a cannulated femoral vein of

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the animal. Inflow and outflow of the bath were exactly matched to prevent alteration of the animal's blood volume.

Group 1. Fourteen metacarpal skin arteries and 11 gracilis muscle arteries were studied in this group. After settng an initial tension of 1000 mg, a strip was allowed to equilibrate in the flowing blood bath until a constant base line was recorded (35 to 80 min).

Serotonin creatinine sulfate (Upjohn Company, Kalamazoo, Michigan) was infused (Harvard Infusion Pump) at 0.5 and 1.0 ml/min (100 μg/ml solution conc) behind the pump. Norepinephrine and acetylcholine or histamine were infused separately at 0.5 and 1.0 ml/min (10 μg/ml solution conc) or injected at 0.5 and 1 ml (10 μg/ml solution conc) behind the pump to test the ability of the strips to contract or relax, respectively.

Group 2. In this group of experiments, artery strips from two different parts of the hindlimb (8 metacarpal skin arteries and 8 gracilis muscle arteries) shared a common bath but were attached to separate force transducers. This made it possible to simultaneously compare the response of vascular smooth muscle from different sources to the same stimuli. An initial vessel strip tension of 250 to 400 mg (14) was established. Approximately the same time was allowed for base line equilibration as in group 1 and the same agents were tested.

Group 3. The same procedure described for group 1 was followed in this group of 8 metacarpal skin and 8 gracilis muscle artery experiments. However, this group differed from the other in that norepinephrine was infused continually throughout the experiment to ensure a degree of active tension in strips. The ability of the strips to relax was tested by injecting acetylcholine or histamine at 0.25–0.5 ml (10 μg/ml). Only vessel strips that responded to acetylcholine or histamine with a decrease in tension were used in this group. Serotonin was then infused during the period of norepinephrine infusion.

Results. Data were analyzed statistically using: (i) the student's t test modified for paired replicates; and (ii) the standard Student's t test for comparison of the means; p values less than 0.05 were considered significant.

Figure 1 represents the average responses

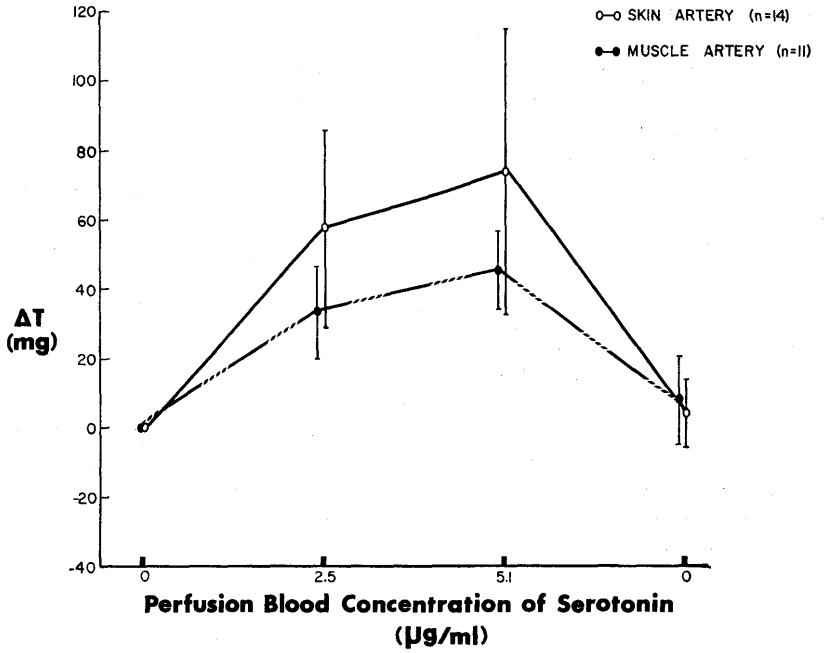

FIG. 1. Effect of serotonin infusion, 2.5–5.1 μg/ml of perfusing blood, of skin and muscle artery atrips in separate baths: n = number of strips; standard error of mean is indicated.

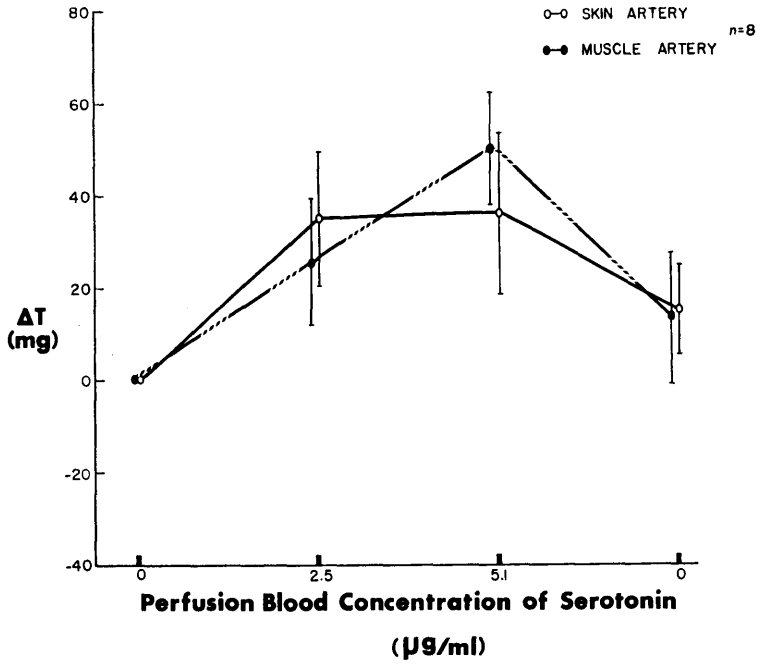

FIG. 2. Effect of serotonin, 2.5–5.1 μg/ml of perfusing blood, on skin and muscle artery strips sharing the same bath: n = number of skin and muscle strips; standard error of the mean is indicated.

of skin and muscle arteries, in separate baths, during serotonin infusion. Mean tension developed by skin arteries was 58 mg during infusion of serotonin at 50 μg/min (2.5 μg/ml of blood) and increased further to about 72 mg at the faster infusion rate ($p < 0.05$). Tension increased by 31 mg in muscle artery strips during infusion at 50 μg/min and rose further to 44 mg when the rate was increased to 100 μg/min ($p < 0.05$). Although the average response of skin strips appeared to be greater than muscle strips, the difference was not significant ($p > 0.05$). These strips all exhibited an increase in tension during norepinephrine administration but did not respond to acetylcholine or histamine.

Figure 2 shows the response of skin and muscle artery strips which shared the same bath to serotonin infusion. Serotonin caused an increase in tension in both types of strips at each infusion rate ($p < 0.05$), but again the response of skin and muscle artery strips was not significantly different ($p > 0.05$). These strips responded similarly to norepinephrine, acetylcholine, or histamine as in the previous group.

Figure 3 represents the average responses of skin and muscle artery strips, in separate baths, when active tension was elicited and maintained by local norepinephrine infusion. Administration of serotonin, histamine, or acetylcholine was superimposed during norepinephrine infusion. Only strips which showed a decrease in tension following local injection of histamine or acetylcholine were used. The left part shows the maximum increase in tension of skin and muscle artery strips which was maintained during norepinephrine infusion. The center portion shows the decrease in tension following injection of histamine or acetylcholine ($p < 0.05$). The right portion shows that both skin and muscle artery strips responded to serotonin infusion with an increase in tension ($p < 0.05$).

Discussion. Results from the present study show that strips of large arteries (1.0–1.5-mm diam) from skin or muscle always respond to serotonin with increases in tension even when the strips respond to acetylcholine or histamine with a fall in tension. This response is similar to those reported by

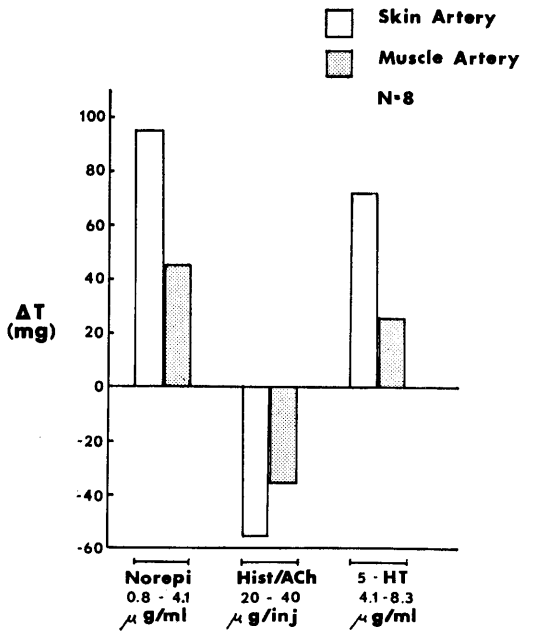

FIG. 3. Response of skin and muscle artery strips, in separate baths, to serotonin and histamine or acetylcholine, superimposed during norepinephrine infusion: n = number of skin and muscle strips.

Bohr and associates in strips of small arterial vessels (200–300-μ diam) from dog cerebral, mesenteric, renal, and pulmonary beds (15, 16) and dog subcutaneous artery (17) under the influence of serotonin. Hence, there are few data to suggest that serotonin has the ability to directly cause relaxation of vascular smooth muscle of isolated vessel strips.

On the other hand, *in situ* studies indicate that serotonin can cause a decrease in total vascular resistance under conditions of high neurogenic tone in the dog forelimb (3), hindlimb, renal, mesentary (4), and gracilis muscle (11) vasculatures. Also, it is clear that in the dog forelimb ia infusion of serotonin always causes an increase in resistance in all segments of skin vessels, whereas skeletal muscle vessels may be little affected or dilate (7–9). It should be emphasized that *in situ* arteries of the size used in the current study *in vitro* respond differently in skin and muscle, *i.e.*, constriction in skin and little if any response in muscle. Comparison of the present study with earlier work from our laboratories and others shows that the response of blood vessel strips isolated from skeletal muscle can be different than the response of *in situ* blood vessels from the same tissue. The difference in response in this situation and also in skin vs muscle blood vessels suggests an indirect action of serotonin on skeletal muscle vessels *in situ*. In this regard, serotonin has been reported to release histamine (1, 18) and to increase the sensitivity of dilator "receptors" or to enhance the effect of stimulation of dilator "receptors" (4). Preliminary bioassay experiments carried out in our laboratory have failed to provide evidence for release of a dilator substance (7). When venous outflow from a gracilis muscle which was being infused ia with serotonin was perfused through an isolated hindpaw, only an increase in resistance was observed in the hindpaw. However, it is possible that a dilator substance, if present, was inactivated before reaching the assay organ or that serotonin overwhelmed any potential dilator action on the assay organ. Further studies along this line in which the constrictor response to serotonin in the hindpaw assay organ was blocked would be beneficial in answering this question.

Summary. Experiments were completed in isolated strips of moderately large arteries from skin and skeletal muscle. The strips were situated in a reservoir through which blood from a dog was continually circulated by means of an extracorporeal system; tension was recorded by means of Statham force transducers and a Grass recorder. The response of the artery strips from both skin and muscle was always an increase in tension during serotonin infusion, even when the same strips relaxed during acetylcholine or histamine administration. Since *in situ* skin vessels always constrict during serotonin administration, while *in situ* medium to large skeletal muscle arteries may exhibit little response to serotonin, this agent may cause some indirect action in the muscle vascular bed.

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