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Regional Blood-Flow Changes during 72-Hour Avoidance Schedules in the Monkey

Abstract. *Systemic and regional blood-flow measurements were made in five restrained monkeys before and during 72 hours of continuous work on an avoidance schedule. Systemic arterial pressures were elevated throughout the stress, initially owing to an increase in cardiac output, and after 72 hours owing to an increase in total peripheral resistance. Changes in the resistance in skeletal muscle blood vessels were closely related to these changes in total peripheral resistance.*

Psychological factors can be a potent influence in the acute elevation of systemic arterial pressure, and it has long been suspected that they play a major role in the pathogenesis of essential hypertension. Although much work has been done on the hemodynamic response to acute psychological stimuli (*1*), there is little information available about the changes in systemic and regional blood flow that occur during prolonged periods of emotional stress or anxiety.

The recent development of the radioactively labeled microsphere method (*2*) has made possible up to five separate measurements of the simultaneous regional blood flow and resistance in the awake, restrained monkey (*3*). This study reports the use of this method to assess the systemic and regional cardiovascular changes occurring during a 72-hour period of work on an avoidance schedule known to elevate urinary levels of 17-hydroxycorticosteroids, epinephrine, and norepinephrine (*4*).

Five male rhesus (*Macaca mulatta*) monkeys, weighing from 3.8 to 4.7 kg, were kept sitting in restraining chairs inside isolation booths for the duration of the experiment. In each animal polyvinyl catheters were surgically inserted into the abdominal aorta below the renal arteries and the inferior vena cava via the external iliac artery and vein, respectively; a third catheter was passed retrograde into the left ventricle through the left common carotid artery. The catheters, brought under the skin to the umbilical area and then to the outside of the isolation booth, were kept patent by a continuous infusion (at 1 ml/hr) of 0.9 percent NaCl solution containing 5 USP units of heparin per milliliter. Arterial, venous, and left ventricular end-diastolic pressures were measured with Statham P23Gb strain gauges placed at the mid-thoracic level and recorded on a Beckman type R Dynograph.

Each time the regional measurements were made the cardiac output was determined in duplicate by the indocyanine-green dye method with a Waters ×301 densitometer. Then, a batch (5000 to 10,000) of plastic microspheres, 50 μ in diameter, labeled with 500,000 to 2,000,000 count/min of different gamma-emitting nuclides (either ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr, or ^{95}Nb), was injected over a 15- to 20-second period through the left ventricular catheter. The spheres mix with blood in the left ventricle and travel with the blood until trapped in the arterioles in the end organs; they do not disturb the circulation since only about 0.1 percent of the total number of arterioles are so blocked. At the completion of the fifth injection the monkey was killed and the major organs plus the remaining tissues (so that total body counts could be obtained) were removed, weighed, and counted (*5*) in glass vials with a Nuclear-Chicago scintillation counter and a calibrated pulse height analyzer which divided the radioactivity into 100 channels of 10 kev. Since each microsphere label has a distinctive gamma-emission spectrum, the amount of radioactivity for each isotope in each organ was determined with appropriate correction factors for the known overlap of the isotope energies.

The fraction of cardiac output to each organ at the time of each of the determinations was the percentage of radioactivity in that organ compared with the sum of the radioactivity of that isotope found in the total body. The blood flow to each organ was the fraction of cardiac output delivered to that organ times the cardiac output determined by dye-dilution. Organ resistance was calculated as the mean pressure gradient $(\bar{P}_a - \bar{P}_v)$/flow to that organ (flow being measured in liters per minute). Details of the procedure, baseline values, and validation data have been described in detail (*2, 3*).

Four to 7 days after the surgical procedure each monkey was trained on a standard Sidman avoidance procedure with a 20-second response-shock interval (*6*). On this schedule the monkey learns to push a lever that resets a 20-second timer which, if allowed to complete its cycle, causes a shock to be delivered through an electrode taped to the monkey's tail. The shock intensity was adjusted to the minimal level which would maintain avoidance behavior. After each animal was trained (from 3 to 5 days) he was allowed from 7 to 10 days of rest prior to the experimental procedure. Pressures from each catheter, cardiac output, regional blood flows, and arterial samples for measurement of hematocrit and blood gases were measured before (baseline), and 20 minutes, 4, 24, and 72 hours after the avoidance schedule was begun. No punishing shocks were delivered for 5 minutes before or during the regional blood-flow measurements.

Previous work has shown that control monkeys have little change in their systemic and regional blood-flow measurements during either a 1-day or 2-day period in which the microsphere and cardiac output measurements were made (*3*). Tests of significance were calculated comparing the changes in the environmentally stressed animals with five control monkeys who had their regional flows measured over a 2-day period. The *t*-test for independent groups was used for the systematic variables and the nonparametric Mann-Whitney U-test for regional changes. Similarly significant levels were found in all variables when the changes in the experimental animals were compared with their baseline measurements. None of the baseline systemic or regional measurements in the experimental group were significantly different from the control group.

Systemic arterial pressures were significantly elevated throughout the work period; systolic, diastolic, and mean arterial pressures remained from 18 to 27 mm-Hg higher than during the baseline period (Table 1). The initial pressor response at 20 minutes and 4 hours was entirely due to consistent rises in both cardiac output and heart rate; stroke volume did not change significantly. At 24 hours the pressures were maintained at a higher level owing to both a slightly (nonsignificantly) higher cardiac output and total peripheral resistance. This trend continued and at 72 hours the elevated pressures were entirely due to a significantly elevated total peripheral resistance; cardiac output and heart rate were not different from baseline levels. There were no significant changes in the experimental, compared to the control, group in central venous or left ventricular end diastolic pressure, stroke volume, arterial PO_2, PCO_2, *p*H, or hematocrit.

The mean percentage changes in cardiac output, total peripheral resistance, and the fraction of cardiac output delivered to nine major organs of the experimental group, compared to their own baseline measurement, are shown in Fig. 1. Changes in regional organ resistance were, in general, similarly significant in the opposite direction as the percentage changes in Fig. 1. As shown in Table 1, the kidneys were the only organ listed that had a significant decrease in blood flow 20 minutes after the stress began; at this time there were large increases of blood flow to the heart, skeletal muscle, and liver (hepatic artery). By 72 hours the pattern

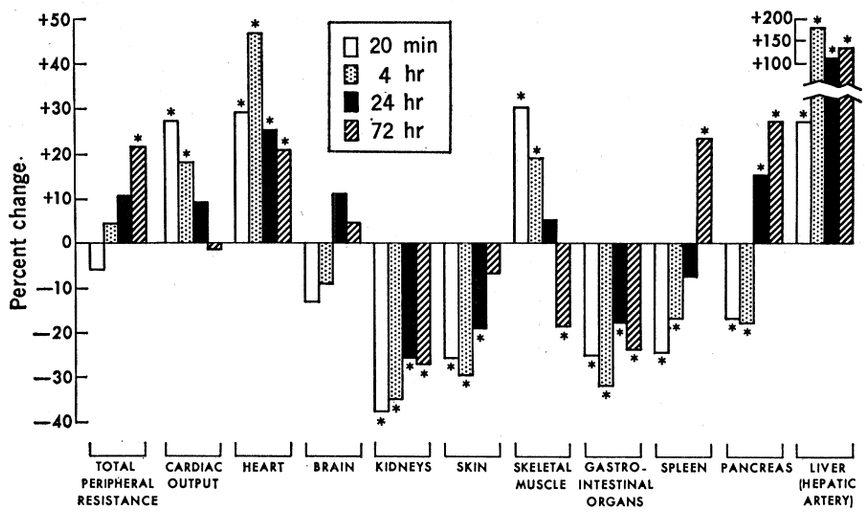

Fig. 1. Mean percentage changes, compared to the baseline measurement, in the total peripheral resistance, cardiac output, and regional fractions of cardiac output in five monkeys in nine major organs during the 72-hour stress period. The gastrointestinal tract includes the stomach, small and large intestines, and cecum. An asterisk indicates significance levels of $P < .05$ compared to control-group changes, by use of the Mann-Whitney U-test. Inset denotes time after initiation of stress.

of redistribution of the cardiac output had changed substantially; blood flow was significantly increased to the heart, spleen, pancreas, and liver (hepatic artery) at the expense of the kidneys, skeletal muscle, and gastrointestinal organs. Blood flow to brain tissue remained unchanged throughout the stress period, indicating the operation of autoregulatory mechanisms. Different portions of the heart, brain, skin, and skeletal muscle, separately dissected and counted, showed substantially the same changes as the whole organ.

Besides the organs represented in Table 1 and Fig. 1, the chest wall and diaphragm showed changes like skeletal muscle, and the mesentery showed changes like pancreas. Samples of fat received a higher fraction of the car-

Table 1. Measurements in the five experimental monkeys.

Variable	Baseline		Stress period							
			20 minutes		4 hours		24 hours		72 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mean arterial pressure (mm-Hg)	102	11	128*	6	123*	11	125*	11	123*	9
Cardiac output (ml/min per kg of body weight)	261	39	352*	67	322*	37	286	39	259	36
Total peripheral resistance [mm-Hg per cardiac output (in liter/min per kg)]	396	14	365	27	382	14	429	31	473*	28
Heart rate (beats/min)	145	18	190*	27	188*	31	187*	32	163	12
Regional blood flows (ml/min per 100 g) in:										
Heart	384	51	559†	43	549†	53	476†	37	473†	42
Brain	81	23	89	22	81	18	95	23	86	17
Kidneys	957	77	807†	43	700†	86	762†	61	782†	60
Skin	24	9	21	8	15†	8	18	5	20	7
Skeletal muscle	27	7	41†	7	33	12	25	3	19†	3
Gastrointestinal organs	87	5	87	12	67†	4	76	9	61†	11
Spleen	277	54	274	63	274	50	288	53	335†	59
Pancreas	281	41	333	58	258	52	334	37	355†	61
Liver (hepatic artery)	40	19	67†	15	99†	23	83†	22	88†	25

* Different from controls at $P < .05$, *t*-test. † Different from controls at $P < .05$, Mann-Whitney U-test.

diac output and increased blood flow at 20 minutes and 72 hours, lower at 4 and 24 hours. The adrenals, bone (limb bone, skull, and spine), and bronchial artery flow to the lung (except at 20 minutes and 4 hours) had a significantly higher fraction of output and lower resistance throughout the stress period; the thyroid had a lower fraction of cardiac output and blood flow and a higher resistance during the stress.

After the initial fall, the continued increase in resistance of skeletal muscle (which in these monkeys comprised 43 percent of the animal's weight) was the predominant regional influence contributing to the increase in total peripheral resistance. Because the amount of muscular activity was not systematically evaluated during the experiment it was not possible to evaluate the metabolic requirements in muscle which may have contributed to the blood flow and resistance changes.

The very large increases in the fraction of cardiac output to the hepatic artery throughout the stress period is similar to that found in bled monkeys (*7*). Although these observed distributional changes do not, necessarily, reflect the nutritional demands of tissues, it seems that the maintenance of arterial blood flow to the liver, as to the heart, is an important aspect of increased sympathetic function.

It can be concluded that a combination of behavioral stress, fatigue, and other physical factors in the primate results in a maintained elevation of systemic arterial pressure over a 72-hour period. Initially, the elevated pressure is due to an increase in cardiac output with a balanced pattern of peripheral resistance changes. Subsequently, cardiac output returns toward baseline levels while total peripheral resistance increases, primarily owing to the progressive vasoconstriction in skeletal muscle. Neurogenic, humoral, and local tissue factors are all probably important for the observed hemodynamic changes, but are at present undefined.

A relationship between long-term avoidance conditioning and high systemic blood pressure in the monkey has been previously demonstrated (*8*). The main contribution of this report is to elucidate the possible hemodynamic mechanisms operating to produce this stress-related hypertension, although the duration and the severity of the stress and related physical factors were of a much different nature in the present study. Further, the preparation and the methods described demonstrate the feasibility of studying the contribution of both humoral and autonomic responses to environmentally induced stress in the unanesthetized primate.

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Contraction of Granulation Tissue in vitro: Similarity to Smooth Muscle

Abstract. *Strips of granulation tissue from three different experimental models contract in vitro when treated with substances that induce contraction of smooth muscle. Because the fibroblasts in such tissues have some ultrastructural features typical of smooth muscle, our findings indicate that fibroblasts are able to modulate toward a cell type that is morphologically and functionally close to smooth muscle.*

The process of repair often results in local shrinkage, best exemplified by the contraction of healing wounds. This shrinkage is thought to depend upon contraction of the granulation tissue (*1*), probably of its fibroblasts (*2*). Indeed, cultured fibroblasts extracted with glycerin contract in vitro in response to adenosine triphosphate (*3*), presumably as the result of an effect on actomyosin (*4*).

In an electron microscopic study of fibroblasts in four models of contracting granulation tissue, we were impressed by the fact that many of these cells developed characteristics intermediate between those of "typical" fibroblasts and those of smooth muscle cells (*5*). This suggested that granulation tissue might contract in response to the same stimuli that affect smooth muscle in vitro.

Therefore, we tested the contractility of strips of granulation tissue as it is routinely tested on smooth muscle. The preparations were suspended from one end of a frontal lever (*6*) and attached to the bottom of a bath containing 20 ml of Tyrode's solution maintained at 37°C and bubbled with 95 percent oxygen plus 5 percent carbon dioxide. The lever amplified vertical displacement by a factor of 6; the tungsten recording stylus inscribed on metallized paper advancing on a kymograph (*6*) at a rate of 1 mm per minute.

We first used granulation tissue from Selye's "granuloma pouch" (*7*), produced in 100- to 130-g male Wistar albino rats by the injection of 20 ml of air and then 1 ml of 1 percent croton oil (*8*) in corn oil into the dorsal subcutaneous tissue. A spiral cut around the pouch wall (1 to 2 mm thick) gave a strip of tissue 70 to 90 mm long and 6 to 8 mm wide weighing 700 to 1100 mg.

5-Hydroxytryptamine (5-HT) (1×10^{-5} g per milliliter of Tyrode's solution, final concentration in the bath) caused an immediate contraction of such strips (Fig. 1A), which usually reached a maximum in 1 to 2 minutes; the contraction tended to persist at this level or relax slightly, but did not return to the baseline. The actual shortening of the tissue was of the order of 3 percent. A typical dose-response study is shown in Fig. 1B. In strips left for 1 hour in the bath without oxygenation, the effect of 5-HT was greatly diminished. The age of the granulation tissue also made some difference; a strip from a 7-day pouch failed to contract with 5-HT, and one from an 8-day pouch contracted only slightly. However, strips from pouches 11 to 32 days old reacted uniformly well, despite the older ones being histologically a little more fibrous. Bradykinin (1×10^{-5} g/ml) caused a similar though lesser contraction than 5-HT at the same concentration; the response