Reflex Circulatory Effects Elicited by Hypertonic and Hypotonic Solutions Injected into Femoral and Brachial Arteries of Dogs

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In the course of study of the circulatory and renal responses to rapid salt-loading in dogs, it was noted that intra-arterial (and intravenous) infusions of small volumes of hypertonic saline solution produced pronounced alterations in the blood pressure and heart rate. Results of infusions into the femoral and brachial arteries of dogs form the substance of the present communication.

The findings are considered to indicate that there is a receptor network in the limbs which is responsive to changes in the osmolarity of the arterial perfusate, and which is capable of reflexly provoking a sympathetic discharge. While the administration of hypertonic solutions intravenously also provokes changes in blood pressure, the details of such observations will not be presented here, but will be offered only to contrast with those obtained by intra-arterial infusion. Circulatory and respiratory responses to intracarotid injection of hypertonic solutions have been demonstrated by Holland, Sundsten and Sawyer. The reaction consists of bradycardia, brief hypotension followed by prolonged hypertension and apnea with the “osmoreceptor” site lying in the medulla.1-6

Circulatory reflexes which are known to originate within the distribution of the femoral and brachial arteries are confined to weak and inconstant baroreceptor activity and vasovagal responses to needle puncture of the artery.7 Reflex respiratory stimulation in dogs following intra-arterial injection of isotonic solutions of phosphate and bicarbonate, of various acids, of KCL added to isotonic saline and of ether has been demonstrated by Comroe and Schmidt9 who consider that this peripheral reflex is essentially related to the mechanism of pain appreciation. Moore, Moore and Singleton in 193310-12 reported the effects of intra-arterial injections of solutions of varying osmolarity. Their observations, however, were not directed toward circulatory measurements, but rather to investigation of pathways of vascular pain as judged by “quasi-emotive” responses. Review of the pertinent literature8-18 has failed to disclose a prior description of a circulatory reflex initiated through the arterial route of a peripherally located “osmoreceptor” system.

**Methods**

Fifty-four adult mongrel dogs of both sexes, weighing between 12 to 25 Kg, were studied. They were anesthetized with sodium pentobarbital (Nembutal) injected intravenously (25 mg./Kg.) and maintained in the anesthetized state by its intermittent administration. Anesthesia was always maintained at a level sufficiently deep to abolish pain sensation, and any operative procedure could be performed without arousing visible evidence of pain.

Blood pressures were recorded through a Courmand needle directed upstream in a femoral artery either punctured percutaneously or cannulated directly after surgical exposure. Statham and Sanborn transducers were employed. Tracings were obtained, either on a direct-writing, 4-channel recorder or on a 6-channel oscilloscopic recorder, at a paper speed of 2.5 mm. per second. The animals were given a single intravenous dose of 40 to 60 mg. of aqueous heparin solution to permit continuous recording of pressures over a protracted period.

Intra-arterial injections were made into the femo-
Figure 1

A. Rise in arterial pressure begins 7 seconds after the onset of the intra-arterial injection (10 ml., 5 per cent saline) followed by cardiac acceleration, increase in pulse pressure and in the depth and rate of respiration (as shown by the respiratory fluctuations of the pressure tracing). B. Absence of response in the same animal to isotonic solution (10 ml.). C. Hypotensive response to distilled water (10 ml.) beginning 4 seconds after injection.

oral and bronchial arteries and their branches. Pressures were always recorded from the limb opposite to the limb into which the injection has been made. Injections were made through Courmand needles directed upstream or downstream in the vessels, through cardiac catheters, size nos. 5 to 8 wedged distally in the popliteal artery, or via a tiny polyethylene catheter (outside diameter = 1.25 mm.) wedged distally in a small branch of the popliteal artery. Five different solutions were injected: saline (0.225, 0.45, 0.9, 1.25, 2.5 and 5 per cent); dextrose (1.25, 2.5, 5, 12.5, 25, and 50 per cent); urea (52.5 and 105 Gm./L.); autogenous blood; and distilled water. The volumes injected varied from 2 to 50 ml. Injections were made by manual pressure using syringes up to 100 ml. in size, and were given with maximal possible speed. Both the onset and duration of injection were recorded. Variations in injection time were small for a given volume of solution. Measurements of pulse rate and respiratory rate were made from the graphic record of the arterial pressure pulse.

Intravenous injections were made into the superficial and deep veins of the upper and lower extremities, and into the inferior vena cava as far up as the right heart. The solutions injected and the method of infusion were identical with those used for the intra-arterial studies.

Three types of denervation experiments were carried out on the lower limbs. Two dogs were subjected to unilateral sympathetic ganglionectomy with removal of the intervening sympathetic chain from T11 to L4 and were allowed 10 to 14 days of convalescence prior to study. Three dogs were studied immediately after partial guillotine-type midthigh transection of 1 lower limb, a procedure which left intact only the femoral artery and vein, stripped of their adventitia, the sciatic nerve, and the femur stripped of its periosteum.

In selected instances, phentolamine methanesulfonate (Regitine) in doses of 2 to 5 mg. was given intravenously immediately before, during, or shortly after intra-arterial injection of hypertonic solutions.

Results

Hypertonic solutions of saline, dextrose and urea, injected into the femoral or brachial arteries, produced an increase in systolic, diastolic, and pulse pressures, heart rate and respiratory rate. Isotonic solutions produced no such circulatory changes. Hypotonic solutions tended to cause small depressions of systolic, and pulse pressures, and mild tachypnea; the heart rate was unaffected (fig. 1).

In general, brachial artery injections provoked circulatory responses of lesser magnitude.

Forty-two femoral intra-arterial injections of 40 to 80 ml. of 5 per cent saline were made in 24 dogs. In every dog and in all but 1 trial, the response was basically as described. The range of blood pressure change in those with a positive response was from 16 to 78 mm. Hg systolic and from 6 to 30 mm. Hg diastolic, with a mean change of 42/20 mm. Hg. The increase in heart rate ranged between 0 and 66 beats/min., with a mean of 20. The increase in respiratory rate was 0 to 300 per cent with a mean of 140 per cent.

The typical response to intra-arterial injection of hypertonic solutions (fig. 1) can be divided into 3 phases. The first phase begins 6 to 10 seconds after onset of injection and lasts 3 to 5 seconds. It is characterized by a rise in diastolic and systolic pressure, small
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decrease in pulse pressure and no change in cardiac or respiratory rate. During the second phase which lasts up to 10 seconds, the diastolic and systolic pressures continue to increase with the latter now exceeding the former, and pulse pressure therefore rises. Cardiac acceleration occurs in the great majority of instances. Hyperpnea begins during this time, with increase in rate and depth of respiration. When respiratory effects are pronounced, usually in response to the solutions of highest concentration, expiratory vocalization may occur. In the third phase, there is a gradual return of circulatory and respiratory dynamics to normal. Control levels, in most instances, are reached in 3 to 8 minutes after onset of injection, but in some cases the baseline is not reached for 30 minutes or more. In a small number of trials, hypotension below basal levels occurred during the third phase.

To test the threshold of the receptor system, saline solutions of graded osmolarity (0.9 to 5 per cent) and identical volume were injected into the flowing femoral arterial stream. No detectable response of blood pressure or heart rate was observed with 0.9 per cent saline in volumes up to 100 ml. while 1.25 per cent saline did cause a definite elevation of pressure. In 10 trial injections in 7 dogs, 10 to 40 ml. of the 1.25 per cent solution provoked a response in 5 of the animals and in 8 of the 10 trials. The average rise of pressure for all trials was 9.7/5.6 mm. Hg with a range of 0 to 24/12 mm. Hg.

Hypotonic solutions produced mild and inconstant hypotension. Eighteen femoral intra-arterial injections of 40 to 80 ml. of distilled water were made into 10 dogs. In 15 trials there was mild hypotension and in 3 no response. Hypertension never occurred. The range of blood pressure change for this group was 0 to 26 mm. Hg systolic and 0 to 23 diastolic, with a mean change of 12/3. The range of respiratory rate increase was 0 to +50 per cent, with a mean of +24 per cent. Similar though lesser changes were observed with 0.22 per cent saline, and occasionally with 0.45 per cent.

Injection of larger volumes of the hypertonic solutions produced effects of greater magnitude. Near maximum effects occurred with 5 per cent saline when volumes of 40 to 50 ml. were used. Definite responses were elicited with volumes as small as 5 ml. of 5 per cent saline.

Femoral artery injections gave quantitatively greater changes than brachial artery injections. In general, with solutions of comparable tonicity, hypertonic saline was most effective followed by dextrose and then urea. Since the pH of stock solution of 5 per cent saline was found to be 5.3 units, bringing it to a pH of 7.4 by the addition of 0.1 N sodium hydroxide prior to injection did not alter the
response curve in 4 trials in 2 animals. That neither transient ischemia nor stimulation of baroreceptor activity were the responsible factors was shown by the fact that complete femoral arterial occlusion for 30 seconds produced no change in systemic blood pressure, whereas the response to hypertonic solutions occurred within 12 seconds. Mechanical distention of the artery with much larger volumes of isotonic solution was without effect. Painful stimuli such as testicular compression and crushing injuries to bone likewise provoked no circulatory response at the level of anesthesia maintained.

In order to study the location of the receptor site, to establish the neural pathway and to eliminate effects which might be ascribed to sudden increase in blood volume or cardiac distention, 3 dogs were prepared with 1 hind limb partially transected leaving intact only the femoral artery, vein, femur and sciatic nerve. This is shown in figure 2. In this preparation, intra-arterial injections of hypertonic and hypotonic solutions provoked their customary response of hypertension and hypotension respectively. If augmentation of the systemic blood volume was prevented by occlusion of the femoral vein during and after the intra-arterial injection, there was no diminution of response. Effects upon the general circulation of purely local vascular response in the injected limb were excluded by making the intra-arterial injection distal to a point of femoral arterial occlusion, and under these circumstances again the typical circulatory responses were obtained. Section of the sciatic nerve in the partially transected limb abolished responses to both hypertonic and hypotonic solutions (fig. 3) regardless of concentration or volume injected. In the intact limb, sciatic nerve section reduced but did not abolish the reflex. The receptor site did not appear to be in the major arteries, for there was no response when the femoral or axillary arteries were filled with hypertonic solutions after having been severed and branches ligated. Moreover, injection of hypertonic solutions through a tiny catheter (1.25 mm. outside diameter) wedged distally in a small branch of the femoral artery provoked an excellent response. Unilateral sympathectomy with removal of ganglia and chain from the eleventh thoracic to third lumbar ganglia failed to diminish materially the response to hypertonic solutions.

In order to ascertain whether circulating catechol amines appeared during this response to intra-arterial injection of hypertonic solutions, the reaction after the intravenous injection of phentolamine (2 to 5 mg.) was studied. In those animals which showed a response to the intra-arterial injection, it was ascertained that the hypertensive response to hypertonic solutions was not abolished by simultaneous injection of phentolamine. However, in 3 of 8 instances phentolamine did cause a pronounced fall in blood pressure when given during the hypertensive phase of the response, suggesting that circulating catechol amines had been released.

A few dogs were given large doses of sodium pentobarbital. In a brief time, they became apneic, markedly hypertensive, areflexic, and no heart beat was discernible on arterial pressure records. When 40 ml. of 5 per cent saline was then injected intra-arterially, there occurred first a striking increase in arterial pressure followed by a return of effective car-

Figure 3
Intra-arterial injection of 40 ml. of 5 per cent saline into partially transected hind limb with sciatic nerve intact. A, showing characteristic response and B, abolition of response following sciatic nerve section.
Intravenous injection of hypertonic solutions resulted in a fall in both systolic and diastolic blood pressures, a rise in pulse pressure, tachycardia, and tachypnea. The response varied directly with osmolarity and/or volume of injected solution, and minimal effects could sometimes be noted with only 5 ml. of 5 per cent saline. The response was identical when injections were made in the superficial limb veins, the deep limb veins, the inferior vena cava, or the right heart. In a partially transected extremity with its sciatic nerve cut, injection of hypertonic solutions intravenously distal to the site of transection reduplicated the typical response. In a partially transected limb, its femoral vein occluded; injection of hypertonic solutions into the femoral vein distal to the site of occlusion failed to elicit a response.

**Discussion**

It has been demonstrated that a pressor response is obtained upon injection of hypertonic solutions into the femoral and brachial arterial systems, is elicited by a variety of substances in hypertonic solution, and increases in magnitude with increasing osmolarity and/or volume of hypertonic injection. Urea solution acts as an excitatory substance because it is presented suddenly in high concentration and establishes a transient extracellular hypertonicity in spite of the fact that it penetrates cells rather freely. On the other hand, intra-arterial injection of isotonic solution does not affect the blood pressure or heart rate and hypertonic solutions tend to decrease blood pressure. These observations suggest that the receptor area behaves as an "osmoreceptor." Thereby, we imply that the receptor is capable of responding to such stimuli, not that it is uniquely sensitive to osmotic stimuli alone.

Studies with intra-arterial injection of solutions distal to a point of femoral arterial occlusion, injections made in the partially transected limb during femoral vein occlusion, and finally the abolition of response by sciatic nerve section in this preparation have shown that the receptor site lies in the limbs and that the afferent pathway is neural and not hormonal.

Preliminary data from the unilateral sympathectomy experiments suggest that the afferent nerve fibers do not enter the spinal cord via the sympathetic ganglia, but, presumably, directly through the dorsal roots.

To our knowledge, this constitutes the first demonstration of an osmotically sensitive cardiovascular reflex, the receptor area of which lies within the distribution of the femoral and brachial arteries. This reflex may be related to that studied by Moore and Moore with reference to the mechanism of arterial pain and by Comroe and Schmidt with reference to reflex respiratory stimulation in dogs.

Transient increases in blood volume cannot be implicated in the etiology of the response as it could be elicited by as little as 5 ml. of 5 per cent saline, while administration of much larger volumes, up to 100 ml., of isotonic solution, were without effect.

Following intra-arterial injection of hypertonic solutions, the brief initial phase (lasting several seconds, wherein diastolic pressure rises more than the systolic, without a change in heart rate) suggests that there is a rise in peripheral resistance. The second phase characterized by an increased systolic, diastolic and pulse pressure, and an increase in the heart rate is best explained as due to cardiac stimulation and an increase in cardiac output. It is during this phase that respiratory stimu-
lation is maximal. The occurrence of circulating catechol amines as part of the response to hypertonic intra-arterial injection was suggested by studies with phentolamine.

The efferent pathway of the response to intra-arterial injection of hypertonic solutions can best be explained as generalized sympathetic discharge. This response is very similar to that provoked by appropriate electrical stimulation of the central cut end of a sciatic or brachial nerve: tachycardia, a rise in blood pressure, and increase in rate and depth of respiration.¹⁹

That the reaction of intra-arterial hypertonic solutions may not merely be the result of a "reaction to pain" as recently defined²⁰ is shown by the fact that the dogs were under deep anesthesia and no effective or circulatory response even to such stimuli as crushing injuries to the toes, testicular compression and partial transection of a leg were elicited. The problem, however, of whether this reflex may be related to the mechanism of pain or is actually operative under the range of physiologic conditions and plays a role in homeostasis remains to be investigated.

The response to intravenous administration of hypertonic saline and dextrose seems quite different from that of intra-arterial. Hypotension rather than hypertension results. The receptor is not peripheral, since responses are obtained equally well in the upper portion of the inferior vena cava and right heart, and no responses are obtained if the solution does not reach the pulmonary circulation. These findings after intravenous injection of small volumes of 5 per cent saline and 25 per cent dextrose solution confirm Eliakim's report²¹ of hypotension following infusion of 20 per cent saline and extend the observation to other hypertonic solutions and to lesser degrees of hypertonicity. The explanation has been offered that the hypotension is due to constriction of pulmonary veins at their entrance to the left atrium.⁸

Summary
Rapid injection of 40 ml. of 5 per cent saline into the femoral or brachial arteries of 24 dogs resulted in an average elevation of arterial blood pressure of +42/+20 mm. Hg, an increase in the heart rate of +20 beats/min., and an acceleration of the respiratory rate of +140 per cent. The response began 6 to 10 seconds after the onset of injection and lasted for 5 to 30 minutes. It occurred with the injection of hypertonic solution of saline, dextrose, and urea. The magnitude of the response was related to the degree of hypertonicity. Responses could be provoked by 10 ml. of 1.25 per cent saline. Rapid intra-arterial injection of isotonic saline, dextrose, and urea solutions, and of whole blood in volumes up to 80 ml. had no circulatory effect. Rapid injection of distilled water, 0.225 per cent and 0.45 per cent saline into the femoral or brachial arteries resulted in a slight degree of hypotension and a rise in the respiratory rate. The appearance of circulating catechol amines following the response to intra-arterial injection of hypertonic solutions was suggested by the demonstration of hypotensive response to intravenous injection of phentolamine. These responses to injection of hypertonic and hypotonic solutions in the femoral artery were abolished by section of the sciatic nerve in animals with an ipsilateral partially-transected hind limb, a preparation which left intact only the femoral artery, femoral vein, and femur. Sciatic section in the intact limb reduced but did not abolish the response. Removal of the sympathetic chain from the eleventh thoracic through the third lumbar ganglia had no apparent effect. It is concluded that the response to femoral and brachial intra-arterial injection of hypertonic and hypotonic solutions is initiated by peripherally located "osmoreceptors" in the distribution of these arteries and is mediated via a reflex whose afferent fibers travel in the peripheral somatic nerves and enter the spinal cord without passing through the sympathetic chain. The efferent arc is the sympathetic nervous system.

Summario in Interlingua
Le rapide injection de 40 ml de un solution salin de 5 pro cento in le arterias femoral o brachial de 24 canes resultava in (1) un augmento medie del tension
RESULTS

De sanguine arterial per 42/20 mm de Hg, (2) un acceleration del frequentia cardiae per 20 pulsus per minuta, e (3) un acceleration del rata respiratorii per 140 pro cento. Le responsa conoenavava 6 a 10 secondas post le initiation del injection e durava inter 5 a 80 minutae. Illo occurreva con le injection del solution hypertonic de sal, dextrosa, o urea, o de sanguine total in volumines de usque a 80 ml. Illo addueva nulle effecto circulatori. Le injection rapid de aqua distillate o de solutiones isotonie de sal, dextrosa, o urea, o de sanguine total in volumines de usque a 80 ml habeva nulle effecto circulatori. Le injection rapid de solutiones hypertonic o de solutiones hypotonic habeva nulle effecto circulatori. Le injection rapid de solutiones hypertonic o de solutiones hypotonic habeva nulle effecto circulatori. Le injection rapid de solutiones hypertonic o de solutiones hypotonic habeva nulle effecto circulatori.

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