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Blood Flow Alteration Induced by Saralasin or Sodium Nitroprusside in Rats

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The radioactive microsphere technique was used to investigate the distribution of blood flow during halothane anesthesia when either sodium nitroprusside (SNP) or saralasin, a competitive inhibitor of angiotensin II, was infused. Seventeen fasted male Wistar rats were anesthetized with halothane and received either saralasin (n = 6) or SNP (n = 11) to decrease mean arterial pressure 20 torr. Cardiac output was unchanged with SNP, but blood flow decreased 23 per cent to the brain, and 25 per cent to the kidney, while splanchnic flow increased 19 per cent ($P < .05$). There were 37 per cent less microspheres present in the lung after drug treatment. Saralasin did not alter cardiac output or flow to other organs but did cause a 49 per cent decrease in the number of microspheres found in the lung after drug treatment. An additional group of rats first received SNP, and then saralasin. This combination was not well tolerated, resulting in lethal hypotension and a mortality of 60 per cent. In the thirteen animals which were able to complete the protocol, increases in blood flow to the heart, kidney and splanchnic circulation were seen while brain flow decreased ($P < .05$). The number of microspheres in the lung also decreased after combined therapy. These studies demonstrate the differential effects of SNP and saralasin in lowering blood pressure. The use of combined drug treatment, when tolerated, may improve organ perfusion. (Key words: Anesthetic techniques: hypotension, induced; nitroprusside. Anesthetics, volatile: halothane. Heart: cardiac output. Measurement techniques: blood flow; microspheres. Pharmacology: nitroprusside; saralasin. Polypeptides: angiotensin.

WE HAVE PREVIOUSLY SHOWN that the renin-angiotensin system exerts considerable control of blood pressure in rats anesthetized with halothane.[1] When saralasin, a competitive inhibitor of angiotensin II is infused, there is a decrease in mean arterial pressure of 20 torr. Sodium nitroprusside also produces a dose-related decrease in blood pressure in rats, and when saralasin is infused in similarly treated animals, a further decrease in blood pressure is observed.[2]

Since blood pressure is influenced by changes in either cardiac output or total peripheral resistance, the modus operandi of the hypotensive effect of SNP or saralasin cannot be discerned from these data alone. More precision in defining the effect of such drug therapy can be achieved through the use of radioactive microspheres. The microsphere technique allows for the determination not only of cardiac output, but also of blood flow to individual organ beds before and after drug treatment. The present study examines the effects of saralasin and SNP-induced hypotension during halothane anesthesia in the rat as a means of providing insight into alterations in distribution of blood flow which occur when these agents are administered.

Methods

Thirty fasted male Wistar rats (270–390 g) were anesthetized with halothane 3–4 per cent in air and maintained with halothane 1.3 per cent inspired. A polyethylene catheter (PE 50) was passed through the right carotid artery and placed in the left ventricle using pressure monitoring. The left femoral artery and vein were cannulated with PE 50 catheters as well. In animals receiving both drugs, a fourth cannula was placed in the right femoral vein. Blood pressure was monitored continuously through the femoral arterial cannula by a Statham P 23 Db pressure transducer using a Brush Mark 260 recorder. All animals were placed under a heating lamp to maintain rectal temperature at 37° C.

To determine cardiac output and distribution of blood flow, carbonized microspheres were used. Strontium-85 (^{85}Sr)- and cerium-141 (^{141}Ce)-labelled microspheres (15 ± 1.1 μM)§ with specific activities of 9.6 mCi/g for ^{85}Sr and 12.8 mCi/g ^{141}Ce, were used. The microspheres had been suspended in dextran 10 per cent, containing Tween 80®, 0.05 per cent. The microspheres were agitated, drawn into a plastic syringe which had been modified to fit into a gamma counting vial, and counted in a Beckman Biogamma® at the appropriate energy spectrum for each isotope. After counting, the microspheres were again agitated, and 0.1–0.2 ml (40,000–60,000 microspheres) were

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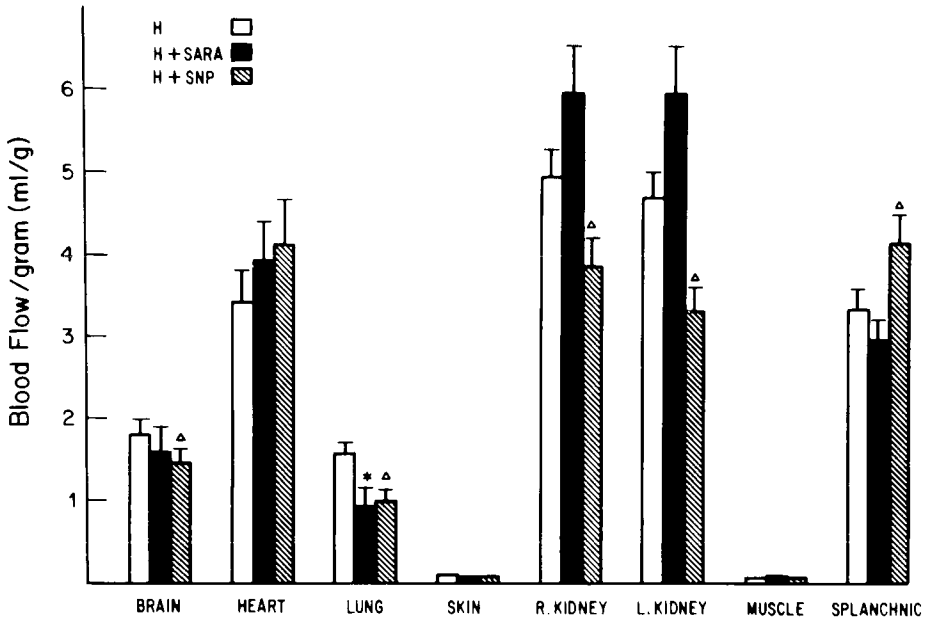

FIG. 1. Blood flow/g of tissue after saralasin (n = 6) or sodium nitroprusside (n = 11) with similar degrees of hypotension (72 ± 6 torr and 69 ± 2 torr respectively). Symbols indicate significant differences ($P < .05$) from paired control value after 1 hour of halothane anesthesia.

injected into the left ventricle catheter over 20 s and flushed with 0.4 ml saline solution. Ten seconds prior to the microsphere injection and for the following 60 s, blood was withdrawn from the femoral artery by a constant-withdrawal Gilford pump. The blood was then placed in a pre-weighed counting vial and the actual withdrawal rate, approximately 0.75 ml/min, was determined. The empty injection syringe was again counted. The above procedure was done for each isotope. Examination of the microspheres by microscopy prior to injection showed no evidence of clumping of the microspheres.

The first injection (^{85}Sr) was done after one hour of stable anesthesia. The rats were then randomly divided into two groups. One group (n = 6) received saralasin, intravenously (100 $\mu g/kg$ for 1 min followed by 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 14 min). The other group (n = 11) received intravenous sodium nitroprusside for 15 min in an individually-titrated dose (ave. 7.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in order to decrease the mean arterial pressure approximately 20 torr. The second injection (^{141}Ce) was done during the last minute of the saralasin or SNP infusion. The animals were then killed by giving potassium chloride through the ventricular catheter.

The organs of the body were removed, weighed, and placed in counting vials. The position of the left ventricular catheter was verified at this time. Samples of skin, muscle, liver, and small intestine were taken; otherwise, the whole organ was counted. Total weights of the liver and small intestine were determined for each animal. The tissue and blood samples were then counted for 5 min at the appropriate energy spectrum, allowing for overlap of strontium in the cerium window.

Cardiac output was determined by the formula: cardiac output = counts injected × reference sample withdrawal rate ÷ reference blood counts. Regional distribution of cardiac output was calculated by comparing the radioactivity in each organ with the total injected radioactivity. Organ flow was determined by multiplying the cardiac output by the fractional distribution of the cardiac output to the organ.

In order to determine whether there were interactions between the vasodilator and the angiotensin II blocker, a third group of rats (n = 13) was studied. These animals were anesthetized with halothane for one hour and then received 7.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of SNP for 15 min. At the end of the SNP infusion, the first set of microspheres was injected (^{85}Sr). The SNP infusion rate was then decreased to 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and saralasin was given for 15 min in a manner similar to the previous group. At the end of this combined drug treatment, the second set of microspheres was injected. The animals were then sacrificed and treated as previously described.

The data presented are the mean values ± standard errors of the mean. The data were analyzed using Student's t test for unpaired data for comparisons between groups and Student's t test for paired data for comparisons within groups. $P < 0.05$ was taken as significant.

Results

After one hour of stable halothane anesthesia, blood pressure, heart rate, cardiac output and blood flow

TABLE 1. Cardiovascular Effects of Saralasin or Sodium Nitroprusside

	Halothane	Halothane + Saralasin (n = 6)	Significance
Blood pressure (torr)	92 ± 6	72 ± 6	$P < 0.05$
Heart rate (bpm)	385 ± 26	370 ± 23	n.s.
Cardiac output (ml/min)	98 ± 11	106 ± 10	n.s.
Peripheral resistance torr/(ml·min^{-1})	0.93 ± 0.13	0.71 ± 0.08	n.s.
	Halothane	Halothane + SNP (n = 11)	Significance
Blood pressure (torr)	88 ± 2	69 ± 2	$P < 0.05$
Heart rate (bpm)	355 ± 16	336 ± 15	n.s.
Cardiac output (ml/min)	95 ± 6	100 ± 5	n.s.
Peripheral resistance torr/(ml·min^{-1})	0.96 ± 0.05	0.70 ± 0.03	$P < 0.05$
	Halothane + SNP	Halothane + SNP + Saralasin (n = 13)	Significance
Blood pressure (torr)	67 ± 3	61 ± 4	$P < 0.05$
Heart rate (bpm)	333 ± 11	340 ± 8	n.s.
Cardiac output (ml/min)	85 ± 5	94 ± 7	n.s.
Peripheral resistance torr/(ml·min^{-1})	0.82 ± 0.06	0.68 ± 0.07	$P < 0.05$

to various organs were similar in the two groups of rats which had received either saralasin or SNP. The data for blood flow/g of tissue therefore were combined and are shown as the control values in figure 1.

With the infusion of saralasin, mean arterial blood pressure declined from 92 to 72 ± 6 torr ($P < .05$). Cardiac output remained unchanged (table 1). Blood flow/g of tissue remained unchanged from the control value except for lung which showed a decreased number of microspheres in the lung tissue (fig. 1). Lung flow represents bronchial flow in addition to the microspheres that were not trapped in other tissues. Renal blood flow was not significantly altered by the saralasin infusion.

Sodium nitroprusside infusion resulted in a decline in mean arterial pressure from 88 to 69 ± 2 torr ($P < .05$). Cardiac output remained unchanged but total peripheral resistance was significantly reduced (table 1). Blood flow to the brain and both kidneys was significantly decreased while splanchnic flow (liver, stomach, spleen, large and small intestine) was increased (fig. 1). A decreased number of microspheres was found in the lung.

Mortality in animals receiving combined drug treatment (SNP and saralasin) was extremely high. After 15 min of SNP, mean arterial pressure was 67 ± 3 torr and cardiac output was 85 ± 4 ml/min. The first microsphere data points for this group (halothane and SNP) were compared to the second microsphere data points (halothane and SNP) for that group which received only SNP therapy and there were no significant differences. When saralasin was added, blood pressure decreased further to 61 ± 4 torr ($P < .05$). (It was at this time that many of the animals developed progressive hypotension and died before the 15 min of saralasin could be completed. These animals were not included in the data). Among the survivors, cardiac output remained unchanged but significant differences in blood flow were seen (fig. 2). There were decreases in blood flow to brain while flow to the heart and viscera was increased. The decrease in renal blood flow which resulted from the SNP infusion was reversed by the infusion of saralasin.

Discussion

The use of radioactive microspheres allows for the determination of cardiac output and the distribution of blood flow before and after drug treatment. We have used this method in the rat to investigate the effects of halothane, enflurane and ketamine anesthesia. We found that many of the changes seen in rats anesthetized with halothane and enflurane were similar to changes known to occur in man.[3]

Previous work from our laboratory has examined the role of the renin-angiotensin system during anesthesia as well as how the renin-angiotensin system contributes to blood pressure support when sodium nitroprusside is infused. We have established that during halothane anesthesia, saralasin, a competitive inhibitor of angiotensin II, could decrease mean blood pressure 20 torr, while plasma renin activity is normal.[1] We have also established that when the renin-angiotensin system is inhibited during SNP-induced hypotension, a further decline in blood pressure is noted.[2] These previous studies, however, did not investigate the mechanism of the fall in blood pressure nor whether important changes in distribution of blood flow were occurring.

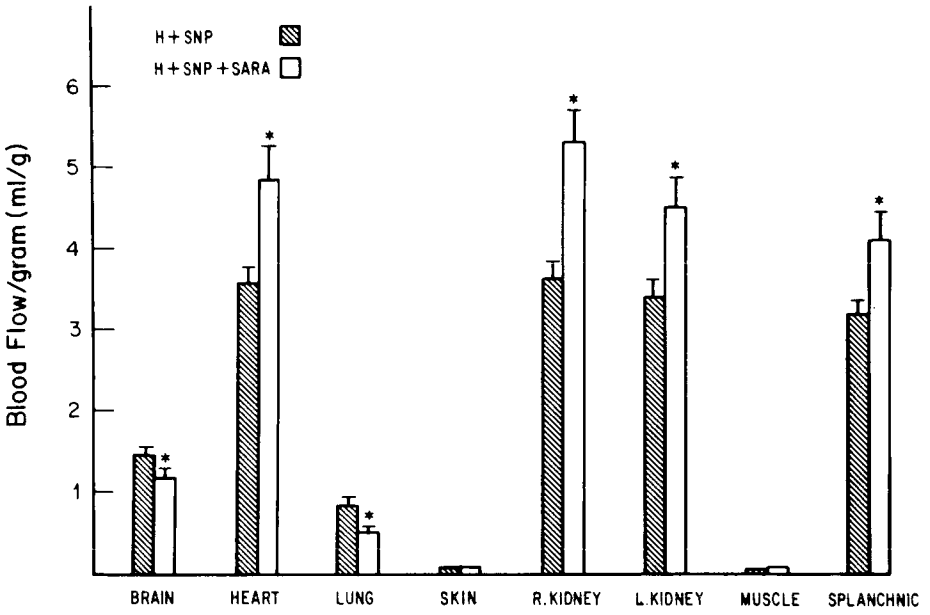

FIG. 2. Effect of sodium nitroprusside and then saralasin on blood flow/g of tissue in rats anesthetized with halothane (n = 13). *Indicates significant differences from the control (halothane and SNP) paired value.

The present experiments using the rat as a model confirm our previous findings. We found that saralasin administration resulted in a fall in blood pressure of 20 torr in rats anesthetized with halothane but that distribution of blood flow was essentially unaltered. The renal blood flow data after saralasin suggest an increase but are not statistically significant. Freeman *et al.* showed that only in states of high plasma renin activity (vena caval construction, sodium depletion) does the infusion of saralasin result in an increase in renal blood flow.[4] In normal dogs, they found no increase in renal blood flow. The decreased blood flow to the lung tissue may represent increased trapping of microspheres by other organs. However, a decreased bronchial blood flow cannot be ruled out in these experiments.

Reports examining total body distribution of blood flow after sodium nitroprusside are limited. Wang *et al.* studied the effects of SNP in dogs anesthetized with pentobarbital and chloralose.[5] Using electromagnetic flowmeters, they found superior mesenteric artery blood flow and coronary flow decreased while renal blood flow was maintained or increased with a mild degree of SNP-induced hypotension (mean arterial pressure 89 torr). These animals had high control heart rates (170 beats/min) suggesting that the control state was one of heightened sympathetic activity. Perhaps this factor as well as species difference explains the differences between our study and Wang's study.

Our data clearly show that splanchnic blood flow is increased with SNP and that renal blood flow is decreased. Bastron and Koloyanides similarly found that in the intact dog, SNP caused a fall in renal blood flow.[6] Behnia *et al.* found that endogenous creatinine clearance was decreased in man during SNP-induced hypotension, again suggesting reduced renal perfusion.[7]

The present study also supports the work of Michenfelder and Theye, who showed that SNP reduced brain blood flow at a cerebral perfusion pressure of 30–40 torr.[8] The insertion of a left ventricular catheter via the right carotid artery might be expected to alter blood flow to the brain. However, studies of Malik *et al.*[9] show that unilateral or bilateral carotid cannulation in the rat does not significantly affect cerebral blood flow or flows to other organs, suggesting that adequate blood flow must exist through the vertebral vessels and other anastomotic channels.

Hypotension, induced by SNP, results in an increase in angiotensin II. This was shown in our previous work. However, the importance of angiotensin II in directing blood flow to various organs is illustrated in these animals who received both SNP and saralasin. First, saralasin infusion resulted in a further decrease in mean arterial pressure and a decrease in brain blood flow. It would appear, therefore, that angiotensin II resulting from SNP-induced hypotension, produces little if any vasoconstriction of the cerebral blood vessels. However, the effects of circulating angiotensin II on other vascular beds are very important. When angiotensin II released under the influence of SNP is inhibited by saralasin, significant increases in blood flow are noted in the heart, kidney, and splanchnic circulation. Angiotensin II is known to be a potent vasoconstrictor of the coronary arteries.[10] Whether the increase in flow is

due only to the inhibition of angiotensin II directly on the coronary circulation cannot be ascertained from the study, but presumably it is a major contributor. It is also known that the renal circulation is exquisitely sensitive to angiotensin II, which when increased in the circulating blood, may be an important controlling mechanism for renal blood flow.[11] Whether inhibition of angiotensin II prior to SNP infusion would be helpful for assuring perfusion of vital organs during hypotension cannot be ascertained from this study. The high mortality associated with the addition of saralasin after SNP infusion has initiated precautions against such use. However, orally effective inhibitors of the renin-angiotensin system (SQ 14,225, Captopril)[12] may be given prior to surgery in patients who will undergo SNP-induced hypotension. Certain vital organs may be better perfused and rebound hypertension may be prevented. When saralasin was infused prior to SNP infusion, admittedly for a short duration, there was no pronounced hypotension nor increased mortality.[13]

The data on splanchnic blood flow are somewhat in conflict when one compares the group receiving SNP alone and the group receiving combined treatment. While there is no statistical difference between the two groups with SNP alone, the absolute value for splanchnic flow in the group ultimately receiving both drugs is lower. When saralasin is administered, a statistical increase in flow is observed but the final value is similar to that found in animals receiving SNP alone. When organ blood flows in individual animals are examined, an explanation for the results is not apparent. We draw no firm conclusions, therefore, concerning the effects of combined treatment on splanchnic flow normally present.

In summary, the influence of saralasin and SNP on various vascular beds has been investigated through the use of the radioactive microsphere technique. SNP decreased brain, lung and renal blood flow while saralasin decreased lung flow only. When sodium nitroprusside and saralasin were infused simultaneously, there was improved coronary and renal perfusion. However, the use of an angiotensin II inhibitor once SNP infusion had begun, resulted in a significant mortality. Perhaps inhibition of the renin-angiotensin system prior to SNP infusion may decrease such mortality and may improve organ perfusion.

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