

Blood Pressure Control during Anesthesia: Importance of the Peripheral Sympathetic Nervous System and Renin

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Newborn rats were treated with guanethidine sulfate for the first three weeks of life in order to produce a partial permanent peripheral sympathectomy. The rats were allowed to grow to 250-300 grams on a normal sodium diet. Using diethyl ether anesthesia, arterial and venous cannulas were placed and the animals allowed to awaken in restraining cages. The rats were divided into four groups: awake ($n = 6$), halothane 1.3 vol % ($n = 8$), enflurane 2.2 vol % ($n = 8$), and ketamine 125 mg/kg, ip ($n = 8$). The protocol consisted of a one-hour control awake period, one hour of stable anesthesia (one group received no anesthesia), and one-half-hour iv infusion of saralasin, a competitive inhibitor of angiotensin II. Plasma renin activity was measured at the end of each time period. Thirty untreated normal rats were similarly divided into four groups and served as the control. The degree of peripheral sympathectomy was assessed through cardiac norepinephrine concentrations, plasma catecholamines, and response to 50% hemorrhage.

Guanethidine treatment resulted in a 78% decrease in cardiac norepinephrine from 189 ± 15 ng/g in the untreated animals compared with 42.4 ± 5 ng/g in the treated animals. The five-fold increase seen in plasma norepinephrine to acute decapitation was completely absent in the treated animals. Hemorrhage of 50% of blood volume resulted in a 75% mortality rate in the treated animals, while there were no deaths 30 min after hemorrhage in the normal animals. Blood pressure for the 30 treated animals during the awake period was 114 ± 2 mmHg, which was significantly less than 124 ± 1 mmHg in the untreated animals ($P < 0.05$). Likewise, plasma renin activity of 1.58 ± 0.25 ng \cdot ml $^{-1}$ \cdot h $^{-1}$ in the treated group was significantly less than 2.59 ± 0.21 ng \cdot ml $^{-1}$ \cdot h $^{-1}$ in the untreated rats ($P < 0.05$). With the induction and maintenance of stable anesthesia, blood pressure decreased to 82 ± 2 mmHg with halothane, 92 ± 4 mmHg with enflurane, and 104 ± 4 mmHg with ketamine in the treated animals. Plasma renin activity did not increase in either treated or untreated animals. Similar degrees of blood pressure decreases were seen in untreated animals. With the infusion of saralasin, a further decrease of approximately 20 mmHg in blood pressure was seen, in both the treated and untreated rats anesthetized with halothane. However, in treated rats anesthetized with enflurane or ketamine, no depressor response to saralasin was seen, which is in marked contrast to the response seen in untreated animals. The plasma renin response in the treated animals also was blunted. Using this animal model, these experiments suggest that partial peripheral sympathectomy does not result in deleterious effects

when rats are anesthetized with halothane, enflurane, or ketamine anesthesia. (Key words: Anesthetics, intravenous: ketamine. Anesthetics, volatile: enflurane; halothane. Polypeptides: renin-angiotensin. Polypeptides: antagonists; saralasin. Sympathetic nervous system: peripheral; guanethidine.)

WITH THE INTRODUCTION of specific inhibitors of the renin-angiotensin system, the importance of angiotensin II in the control of blood pressure in both normal and pathologic states has been quantitated.¹ Our laboratory has used saralasin, a specific angiotensin II competitive inhibitor, to determine what role angiotensin II contributes to blood pressure control in rats anesthetized with various clinical anesthetics. Our results show that during halothane, enflurane, and ketamine anesthesia, a significant depressor effect by saralasin can be demonstrated.^{2,3}

Certainly, however, there are many other control systems which also are involved in blood pressure regulation. The most widely investigated is the sympathetic nervous system. During anesthesia, many of the cardiovascular changes seen have been attributed to their action on the sympathetic nervous system. Shovsted and associates believed that anesthetics affect two centers in the medulla oblongata—a tonically active pressor center and a depressor area excited only by afferent impulses from peripherally located baroreceptors. Both centers act via descending pathways synapsing with spinal vasomotor neurons.⁴ In contrast, Wang and co-workers, using cross-perfusion techniques, suggested that halothane exerted its major effect on peripheral sites.⁵

Recently, techniques have been developed which allow for the pharmacologic dissection of the sympathetic nervous system into peripheral and central components.^{6,7} This experimental technique allows for the evaluation of the peripheral sympathetic nervous system in rats who are awake initially and then anesthetized with various anesthetics.

Using this technique, as well as methods to evaluate the renin-angiotensin system, we have investigated the interaction of the renin-angiotensin system and the peripheral sympathetic nervous system and their roles in blood pressure control when rats are anesthetized with commonly used anesthetic agents.

Methods

Pregnant Wistar rats were housed in individual cages. One week after birth, the newborn rats were treated

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for five days per week for three weeks with guanethidine sulfate ($50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). The guanethidine sulfate was adjusted to a pH 7.0–7.5 and administered subcutaneously in a volume of 5–10 $\mu\text{l/g}$ body weight as suggested by Johnson and co-workers.⁶ After weaning (20–30 days), the rats were separated according to sex and housed in standard cages with full access to food and water. The rats were allowed to grow to 250–300 g size (12–15 weeks) before experimentation was done. All animals were on a normal sodium diet.

Fifty rats survived the drug treatment protocol. These rats were briefly anesthetized with diethyl ether, and a femoral artery and vein cannulated with PE-50 tubing. The cannulas were exteriorized and flushed with a solution of heparin and physiologic saline. The rats were placed in restraining cages for at least one hour in order to recover from anesthesia. Blood pressure, monitored continuously through the arterial cannula by a Statham® P23 Db pressure transducer using a Brush Mark® 2400 recorder, had stabilized well before the end of the recovery period. A group of 30 Wistar rats (250–300 g) which had not received guanethidine served as the control groups for comparison between normal and guanethidine-treated animals.

The protocol consisted of a one-hour control period, a 20-min induction period, a one-hour period of stable anesthesia, and a 30-min infusion of saralasin. Anesthesia was established with one of the following agents: 1 MAC halothane, 1.3 vol %; 1 MAC enflurane, 2.2 vol %; or ketamine, 125 mg/kg, intraperitoneally. A control guanethidine-treated group was treated identically but remained unanesthetized throughout. All animals breathed room air spontaneously throughout the experiment. Inhaled concentrations of the volatile agents were determined at 15-min intervals by gas chromatography. Ketamine was supplemented with half of the initial anesthetic dose after one-half hour of stable anesthesia. This was done to prevent purposeful movements that would be expected to occur approximately 40 min after the initial injection in normal animals. All animals were placed under a heating lamp to maintain rectal temperatures at 37° C.

In order to ensure a large excess of saralasin relative to angiotensin II at the angiotensin binding sites, a loading dose of saralasin, 100 $\mu\text{g/kg}$, dissolved in 0.9% saline solution, was infused intravenously, and the infusion of saralasin was maintained at 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for the next 30 min.⁸ The volume of saralasin administered did not exceed 0.8 ml.

Arterial blood (0.5 ml) was drawn immediately prior to the anesthetic period, and again after one hour of stable anesthesia, for determination of plasma renin activity. A third arterial blood sample was obtained at the end of the saralasin infusion for determination of plasma

renin activity. An equal volume of saline solution was administered intravenously to replace the shed blood. Plasma renin activity was estimated using 0.2 ml plasma by a slight modification of the procedure described by Haber *et al.* using New England Nuclear reagents.⁹ The plasma was incubated for two hours to generate angiotensin I, which was estimated by radioimmunoassay, and the renin activity calculated as $\text{ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$. The plasma samples from the four groups were assayed randomly in ten separate renin assays. The standard curve was done in triplicate while all samples were done in duplicate. Renin values that were high were diluted and reassayed.

In order to assess adequate depletion of catecholamines, three different experimental designs were employed. First, six normal and eight guanethidine-treated rats were killed by decapitation, and cardiac norepinephrine concentrations determined by fluorescence analysis of trihydroxyindoles.¹⁰ Second, because adrenal gland epinephrine is not influenced by guanethidine treatment, six normal and ten guanethidine-treated rats were acutely adrenalectomized. They were allowed to awaken in restraining cages, control blood pressures taken for 30 min, and then hemorrhaged to 50% of their blood volume (7 ml/100 g). Last, six guanethidine-treated and six normal rats were decapitated and the blood from the trunk collected and assayed for catecholamines.

Blood samples for analysis of catecholamines involved collection in iced polystyrene tubes containing EDTA. After centrifugation, 500 μl of plasma were collected and stored at -80°C for later analysis. Catecholamines were extracted 1–10 days later and then assayed by high performance liquid chromatography following a modified procedure of Davis and Kissinger.¹¹ Briefly, 8 pmol of internal standard (3,4-dihydroxybenzylamine) was added to the plasma and the catecholamines were absorbed to alumina under alkaline conditions. The catecholamines then were eluted with 250 μl of 0.1 N perchloric acid; 200 μl of this eluate was injected and then separated by an ODS 5- μm reverse phase column. Catecholamines were detected electrochemically with a 50-pg sensitivity threshold.

The data presented are the mean values \pm standard error of the mean. Statistical significance of the results was determined using one-way analysis of variance among groups and the Student's *t* test for paired or unpaired data when appropriate. $P < 0.05$ was considered significant.

Results

The initial series of newborn rats that were treated had a high mortality rate, but once care was taken in handling the newborn rats with gloves prior to treat-

TABLE 1. Effect of Acute Decapitation on Plasma Catecholamines

	Norepinephrine (ng/ml)	Epinephrine (ng/ml)	Dopamine (ng/ml)
Normal rats (n = 6)			
Before decapitation	0.42 ± 0.11	0.16 ± 0.11	0.09 ± 0.03
After decapitation	2.63 ± 0.72*	0.14 ± 0.06	0.23 ± 0.11
Guanethidine-treated rats (n = 6)			
Before decapitation	0.52 ± 0.06	0.13 ± 0.04	0.08 ± 0.02
After decapitation	0.38 ± 0.07	0.12 ± 0.04	0.10 ± 0.04

* $P < 0.05$.

ment, as well as assuring that body temperature remained normal at the time of drug treatment, the mortality rate decreased to less than 10%. Some of the animals experienced mild diarrhea and ptosis, but not all animals exhibited this, nor did they differ in hemodynamic measurements from animals which did not exhibit these signs.

Measurement of cardiac norepinephrine demonstrated that the six untreated animals had 189.8 ± 14.8 ng of norepinephrine per gram of cardiac tissue. The eight guanethidine-treated animals had 42.4 ± 5.3 ng/g ($P < 0.05$) which represents a 78% reduction in cardiac norepinephrine.

The hemorrhage experiments in the adrenalectomized rats demonstrated significant differences between the two groups. The mean blood pressure for the awake guanethidine-treated group was 107 ± 5 mmHg, while in the normal animals, it was 124 ± 3 mmHg. With 50%

hemorrhage, 75% of the guanethidine-treated animals died either directly or shortly thereafter, while all of the control animals were alive 30 min after hemorrhage.

The decapitation experiments also showed marked differences in the two groups. While there were no differences in plasma catecholamines in either group awake, with decapitation the normal animals had a five-fold increase in plasma norepinephrine, while the guanethidine-treated animals showed no such increase ($P < .05$) (table 1). No differences were observed in epinephrine or dopamine before or after decapitation in either group.

In the guanethidine-treated animals during the awake period, blood pressure and plasma renin activity for the four groups were similar when compared by analysis of variance and, therefore, their results were combined. The mean arterial blood pressure for the 30 guanethidine-treated animals was 114 ± 2 mmHg, and plasma renin activity was 1.58 ± 0.25 ng · ml⁻¹ · h⁻¹. When the normal animals were compared to the guanethidine-treated group during the awake period, both mean arterial blood pressure and plasma renin activity were significantly different. Mean arterial pressure for the 30 normal animals was 124 ± 1 mmHg ($P < 0.05$), while plasma renin activity was 2.59 ± 0.21 ng · ml⁻¹ · h⁻¹ ($P < 0.05$).

In the awake controls for both the normal rats and the guanethidine-treated rats, small differences were seen. During the second hour of wakefulness, plasma renin activity decreased slightly in the guanethidine-treated animals when compared to normal animals. With the infusion of saralasin, a small pressor effect was seen which was significant only in the normal animals (figs. 1-4).

The introduction of halothane anesthesia resulted in significant decreases in blood pressure in both groups. A small but insignificant rise in plasma renin activity was seen in both groups. With the infusion of saralasin, a significant decrease in blood pressure and a significant increase in plasma renin activity was seen in both groups.

The response to enflurane anesthesia differed in the two groups. In the normal animals, blood pressure de-

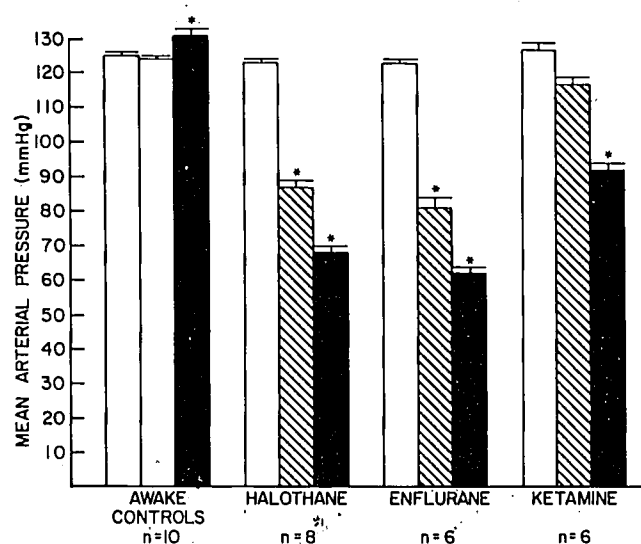


FIG. 1. Mean arterial blood pressure in normal untreated Wistar rats during one hour of an awake control period and during one hour of stable anesthesia with either halothane 1.3 vol %, enflurane 2.2 vol %, or ketamine 125 mg/kg ip. After a 30-min infusion of saralasin, blood pressure decreased in all anesthetized animals. * $P < 0.05$ by paired analysis to previous value. □ = awake; ▨ = anesthetized; and ■ = anesthetized + saralasin.

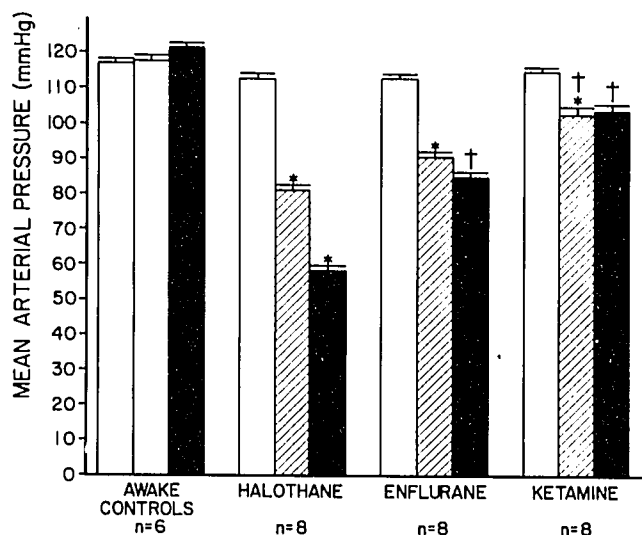


FIG. 2. Mean arterial blood pressure in guanethidine-treated Wistar rats during one hour of an awake control period and during one hour of stable anesthesia with either halothane 1.3 vol %, enflurane 2.2 vol%, or ketamine 125 mg/kg ip. After a 30-min infusion of saralasin, blood pressure decreased only in rats anesthetized with halothane. * $P < 0.05$ by paired analysis; † $P < 0.05$ comparison of untreated *versus* treated rats by unpaired analysis. □ = awake; ▨ = anesthetized; ■ = anesthetized + saralasin.

creased with enflurane and more so with the addition of saralasin. Plasma renin activity increased substantially after saralasin. In contrast, the blood pressure in the guanethidine-treated animals did not decrease as much with enflurane anesthesia, nor was a significant decrease in blood pressure seen with the saralasin infusion. Similarly, plasma renin activity was lower after enflurane anesthesia with the saralasin infusion (figs. 1–4).

The response to ketamine anesthesia also differed in the two groups. In the untreated rats, blood pressure tended to decrease but was significant only after the infusion of saralasin which resulted in a significant increase in plasma renin activity. In the treated animals, a small decrease in blood pressure was seen with ketamine, but the depressor response to saralasin was not present. Plasma renin activity was not as high during anesthesia, and it did not increase significantly after saralasin (figs. 1–4).

Discussion

Over the past 20 years, two methods have been used to produce a permanent sympathectomy in small laboratory animals: immunosympathectomy through the use of an antibody to nerve growth factor and administration of 6-hydroxydopamine. Both of these methods have major disadvantages because the sympathectomy is relatively incomplete,¹² and peripheral administration of 6-hydroxydopamine results in permanent alterations of noradrenergic neurons in the central nervous system.¹³

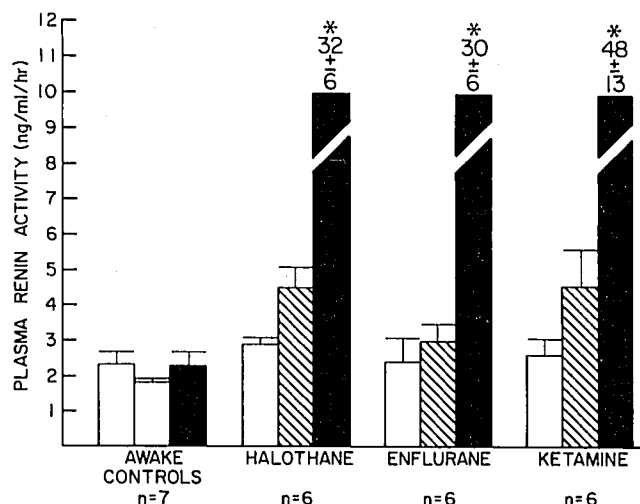


FIG. 3. Plasma renin activity ($\text{ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$) in normal untreated Wistar rats during one hour of an awake control period and during one hour of stable anesthesia with either halothane 1.3 vol %, enflurane 2.2 vol %, or ketamine 125 mg/kg ip. After a 30-min infusion of saralasin, plasma renin activity increased in all anesthetized animals. * $P < 0.05$ by paired analysis to previous value. □ = awake; ▨ = anesthetized; and ■ = anesthetized + saralasin.

The method devised by Johnson and co-workers^{6,7} has the following advantages: a low mortality rate, an 80–90% depletion of peripheral catecholamines while central nervous system catecholamine content remains

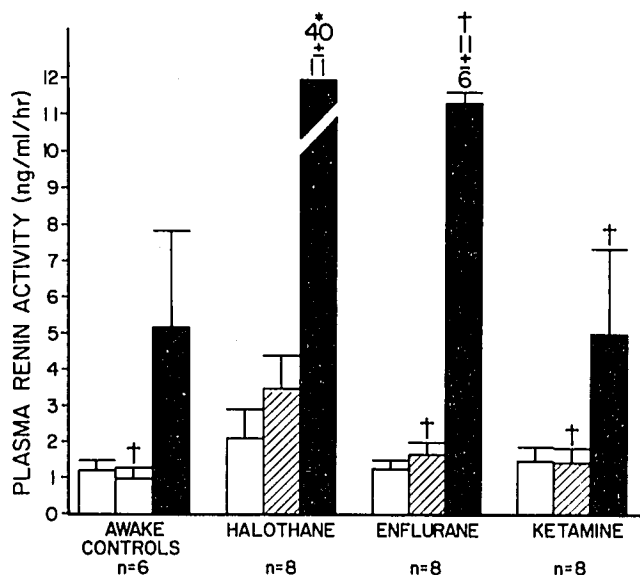


FIG. 4. Plasma renin activity ($\text{ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$) in guanethidine-treated Wistar rats during one hour of an awake control period and during one hour of stable anesthesia with either halothane 1.3 vol %, enflurane 2.2 vol %, or ketamine 125 mg/kg ip. After a 30-min infusion of saralasin, plasma renin activity increased only in animals anesthetized with halothane. * $P < 0.05$ by paired analysis; † $P < 0.05$ comparison of untreated *versus* treated rats by unpaired analysis. □ = awake; ▨ = anesthetized; and ■ = anesthetized + saralasin.

normal, morphologic evidence of destruction of sympathetic ganglia, and marked impairment of the peripheral sympathetic nervous system when stimulated either electrically or pharmacologically.

We rigorously followed this technique and similarly found an approximate 80% reduction in cardiac norepinephrine content. The rats resembled those described by Johnson. Johnson also found the blood pressure to be lower in animals treated with guanethidine than in normal animals. All his measurements, however, were made during pentobarbital anesthesia. In these experiments, the mean arterial blood pressure for all of the rats during the awake period was 114 ± 2 mmHg which is significantly less than 124 ± 1 mmHg for the 30 normal rats.

The decapitation experiments demonstrated that while the circulating amounts of norepinephrine were normal in both groups, the acute stress of decapitation resulted in an increase in norepinephrine only in the untreated animals. This must reflect the fact that guanethidine treatment does not result in complete loss of norepinephrine. When the animal is stressed further, as was done in the 50% hemorrhage experiments, the role of the peripheral sympathetic nervous system in blood pressure support became most evident. The data indicate that the method of Johnson and co-workers results in a substantial reduction in the peripheral sympathetic nervous system, but its dysfunction is seen only with moderate to severe stress.

This partial ablation of the peripheral sympathetic nervous system did not result in an enhanced response from the renin-angiotensin system in the awake state. Actually, plasma renin activity was lower and the response to saralasin was similar, *i.e.*, a small pressor response. The decreased plasma renin activity perhaps reflects the decreased sympathetic tone.

The blood pressure response in either group of rats anesthetized with halothane was similar. With the addition of saralasin, an approximate 20 mmHg decrease in blood pressure occurred. This further decrease in blood pressure resulted in dramatic increases in plasma renin activity in both groups. This demonstrates that the negative feedback of angiotensin II upon renin release remained intact in animals with partial peripheral sympathectomy.

The response to enflurane anesthesia in the normal animal is similar to our previous study.² The infusion of saralasin results in an approximate 20 mmHg decrease in blood pressure. However, the infusion of saralasin to the treated animals anesthetized with enflurane demonstrated only a small decrease. Also, plasma renin activity did not increase in a manner similar to the untreated animals. The data suggest that the renin response to enflurane anesthesia was attenuated in the

treated animals, and, therefore, the response to saralasin also was attenuated.

The cause of the decreased renin response is unclear from these experiments. The renin response to saralasin in treated rats anesthetized with halothane was normal, suggesting that renin content and releasing mechanisms were normal and intact. Sympathetic stimulation is known to cause renin release.¹⁴ Perhaps the combination of the ablated peripheral sympathetic nervous system, as well as the possible decreased sympathetic tone due to enflurane,¹⁵ results in decreased renin release.

The differences in response of the renin-angiotensin system to halothane and enflurane anesthesia cannot be explained fully by these experiments. Our previous work shows that halothane decreases blood pressure in the rat by a reduction in cardiac output, while enflurane decreases blood pressure by a decrease in peripheral vascular resistance.¹⁶ Cardiac output was not measured in these experiments. However, if cardiac output were increased in the treated animals to compensate for the possible decreased peripheral resistance, then the differences in halothane and enflurane may be explained. Enflurane anesthesia would result in small changes in total peripheral resistance, resulting in a smaller decrease in blood pressure than normally seen. This smaller decrease in blood pressure would result in less renin release and, therefore, less of a response to saralasin. Halothane, in contrast, would decrease the elevated cardiac output, resulting in a large decrease in blood pressure. Since total peripheral resistance is little affected by halothane, the influence of saralasin would be greater and blood pressure would fall further. A similar explanation could be applied to the differences seen between treated and untreated animals when they were anesthetized with ketamine.

In summary, animals which have been raised from birth with a blunted peripheral sympathetic nervous system respond to halothane anesthesia in a manner similar to normal animals. However, with enflurane and ketamine anesthesia, differences in response of blood pressure and the renin-angiotensin system are seen. Perhaps deleterious effects of these anesthetic agents would only be apparent with 100% depletion of the peripheral sympathetic nervous system. The level at which the peripheral sympathetic nervous system dysfunction becomes critical is not precisely defined in these experiments. It would appear, however, that these anesthetics do not act as a severe stress. The treatment of newborn rats with guanethidine allows new investigation of the importance of the peripheral sympathetic nervous system in cardiovascular homeostasis during anesthesia and surgery.

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