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Cardiovascular Effects of Anatoxin-A in the Conscious Rat

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Cardiovascular Effects of Anatoxin-A in the Conscious Rat. SIRÉN, A.-L., AND FEUERSTEIN, G. (1990). *Toxicol. Appl. Pharmacol.* **102,** 91–100. The effects of anatoxin-A on mean arterial pressure (MAP), heart rate, cardiac index (CI), and blood flow (BF) in hindquarter (HQ), renal (R), and mesenteric (M) vascular beds were studied after intravenous (iv) and intracerebroventricular (icv) administration in the conscious rat. The pharmacological profile of anatoxin-A was further compared to nicotine administered iv and icv. MAP and heart rate were measured from femoral artery, CI by thermodilution method, and blood flow by Doppler velocimetry. Anatoxin-A and nicotine (30, 100 and 300 μg/kg iv) produced an increase in MAP with concomitant bradycardia. The highest doses increased CI. MBF and RBF decreased due to a vasoconstriction in M and R vasculature. These effects were attenuated by the ganglion blocker chlorisondamine (5 mg/kg, iv). Anatoxin-A (100 μg/kg, iv) increased plasma epinephrine levels by 2-fold with virtually no effect on norepinephrine whereas nicotine (100 μg/kg, iv) increased plasma epinephrine and norepinephrine by 20- to 30-fold. Central administration of anatoxin-A and nicotine (30–100 μg/kg icv) increased MAP with no effect on heart rate and produced M and R vasoconstriction. In summary, the present study demonstrates that anatoxin-A acts as a nicotinic cholinergic agonist in the conscious rat after both systemic and central administration. Anatoxin-A and nicotine produced pressor and reno-splanchnic vasoconstrictor responses and at high doses increased cardiac output. These effects were mediated by activation of the nicotinic receptors in the adrenal medulla and sympathetic ganglia. However, marked differences were found in the potency of anatoxin-A versus nicotine to stimulate the sympathoadrenomedullary axis.

Anatoxin-A is a natural toxin isolated from the blue green algae Anabena flos-aquae (Devlin *et al.*, 1977). This toxin is produced in freshwater algal blooms and is responsible for the death of livestock and waterfowl via a depolarizing blockade of neuromuscular transmission and subsequent respiratory paralysis (Carmichael *et al.*, 1975). Anatoxin-A is found to be a stereospecific agonist of nicotinic receptors in frog muscle and torpedo electric organs (Swanson *et al.*, 1986). It has also been shown to act upon ganglionic muscarinic and nicotinic receptors of the guinea pig ileum (Carmichael *et al.*, 1979) and to bind to nicotinic cholinergic receptors in the rat brain homogenates (Zhang *et al.*, 1987). In the present study we evaluated whether anatoxin-A will produce similar effects to those of nicotine also in an *in vivo* system. Therefore the effects of anatoxin-A and nicotine on cardiac function and regional blood flow were studied after systemic (intravenous) and central (intracerebroventricular) administration in the conscious rat.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (300–360 g) were purchased from Taconic Farms (Germantown, NY) and housed at 22°C with a 12-hr/12-hr light/dark cycle. After surgical operations the rats were housed individually in

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plastic cages (21 × 27 × 16 cm, W × L × H) with food and water *ad libitum*.

Measurement of cardiac output. The cardiac output was measured by thermodilution technique as previously described in detail (Sirén *et al.*, 1988). Each rat to undergo cardiac output measurements was anesthetized with an intramuscular injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) and a PE-50 catheter was inserted into the left femoral artery. The catheters were tunneled under the skin and exited at the nape of the neck. An incision was made at the midline of the neck from the cricoid to the clavicle, and a PE-50 catheter was inserted into the right atrium via the external jugular vein. The left common carotid artery was exposed and ligated, and a thermistor (MX2-780-33 Model THMP f 1.5, Teflon reusable, Columbus Instruments, OH) was advanced through the carotid into the ascending aorta. Placement above the aortic valve was confirmed in each animal by the shape of the dilution curve before the probe was finally sutured to the neck muscles and again at the end of experiment. The jugular vein catheter and the thermistor leads were tunneled under the skin to the nape of the neck. All catheters and the probe wire were secured by a soft spring wire attached to the animal's neck using an adhesive collar. Twenty-four hours after the surgery, the arterial line was connected to a pressure transducer (Narco Bio-Systems Model RP 1500i) attached to a strain gauge coupler (Narco Bio-Systems Type 7032). Blood pressure (mean, systolic, diastolic, pulse) and heart rate were continuously recorded on a Narcotrace 80 computerized physiograph and sampled automatically at 30- to 60-sec intervals by a Northstar–Hazeltine computer.

To obtain measurements of cardiac output, the aortic thermistor was attached to the computerized Cardiomax II (CMX2-780-k with microprobe option R, Columbus Instruments, OH). The dead space of the venous line was first flushed with 50 μl of 0.9% (w/v) NaCl (saline) at room temperature (22°C). After a brief stabilization period an additional injection of 200 μl normal saline (22°C) was rapidly injected into the right atrium using a 1-ml syringe, and the blood temperature was monitored by the aortic thermistor. The thermodilution curve representing the change in aortic temperature was then calculated by the Cardiomax II. A control period of 15 min included two or three cardiac output recordings to test for consistency and placement of the probe. During this time period, control values for blood pressure and heart rate also were collected. The timer for automatic data collection was started and data points for blood pressure, heart rate, and cardiac output were collected immediately before and 1, 5, 15, 30, and 45 min after anatoxin-A or nicotine injection into the jugular catheter. Total peripheral resistance (TPR) was calculated by dividing the mean arterial pressure by the cardiac output and values of cardiac output and TPR were further indexed per unit of weight (kg). Core temperature was monitored by the aortic thermoprobe before each cardiac output measurement.

Measurement of organ blood flow. Regional blood flow in hindquarter, renal, and mesenteric arteries was monitored in a separate group of rats by using the directional pulsed Doppler technique. Although it does not allow for quantitative blood flow monitoring, this method is superior to other available techniques, since (1) it can be used chronically in conscious animals, (2) it allows continuous on-line recording of blood flow, and (3) it can detect instantaneous, transient changes in blood flow within seconds after drug administration. Haywood and coworkers (1981) demonstrated that the velocity signals recorded from the Doppler flow probes are directly and reliably proportional to changes in true volume flow measured by electromagnetic flowmetry.

Each rat to undergo regional hemodynamic measurements was anesthetized with ketamine–acepromazine, and a guide cannula for icv injections was placed on the skull as described below. A midline laparotomy was then made, and the left renal and superior mesenteric arteries and lower abdominal aorta above its bifurcation were carefully isolated under a dissecting microscope. Doppler flow probes (Valpey–Fisher, Hopkinton, MA) were then loosely sutured around each vessel as described earlier (Haywood *et al.*, 1981; Sirén and Feuerstein, 1986; Sirén *et al.*, 1988). The insulated wire leads were fixed to the back muscles, tunneled under the back skin to exit at the neck, and soldered to a receptacle which was then attached to the skull using small screws and dental acrylic. The animals were allowed to recover from the surgery for 7 days. Twenty-four hours before the experiment the rat was reanesthetized with halothane (2% in oxygen) and femoral artery and vein were catheterized with PE-50 tubing. The catheters were tunneled under the back skin, exited at the nape of the neck, and secured by a soft spring wire as described above.

On the day of the experiment, the arterial catheter was connected to a pressure transducer (Narco) and blood pressure and heart rate were continuously recorded on the Narcotrace 80 physiograph. A cable connecting the blood flow receptacle and the Doppler flowmeter (University of Iowa, Bioengineering Facility, Model 545C-4) was attached to the animal and the mean blood flow continuously recorded on the physiograph. Vascular resistance was calculated by dividing the mean arterial pressure by blood velocity (Doppler shift in kilohertz) as earlier described (Haywood *et al.*, 1981; Sirén *et al.*, 1988; Sirén and Feuerstein, 1986). Changes in blood flow and vascular resistance are expressed as a percentage of control values.

Intracerebroventricular injections. Rats were anesthetized with ketamine–acepromazine (see above) and placed on a stereotaxic device (David Kopf Instruments, CA). A stainless-steel guide cannula was inserted through the skull and fixed with glue (Eastman 910 adhesive). Coordinates for the injections into the right lateral brain

ventricle (icv) were measured from the bregma: AP = −0.8 mm and L = 1.2 mm. After the placement of the icv cannula, implantation of Doppler flow probes and femoral catheters was done as described in detail in the previous paragraph (Measurement of organ blood flow). On the day of the experiment, drug injections were made using a premeasured 30 g (7.5 mm) cannula inserted into the ventricular space through the guide cannula. The injection cannula was then connected via polyethylene tubing to a Hamilton microliter syringe, and a volume of 10 μl of the control or drug solution was injected over a period of 30 sec. The proper position of the icv cannula was determined at the end of each experiment by an injection of dye (methylene blue) into the cerebral ventricles.

Assay of plasma catecholamines. Blood samples (0.8 ml) were withdrawn from the arterial catheter 30 min after the iv administration of anatoxin-A or nicotine (100 μg/kg). The blood withdrawn was replaced with an equal volume of heparinized fresh rat blood (10 U.S. units/ml blood). The blood specimens were collected in chilled test tubes and centrifuged (Beckman microfuge B) for 1 min, and the plasma was removed and rapidly frozen on dry ice. Epinephrine (EPI) and norepinephrine (NE) were separated by alumina extraction and assayed by high-performance liquid chromatography with electrochemical detection (Goldstein *et al.*, 1985; Eisenhofer *et al.*, 1986).

Statistical analysis of the data. Data in text and figures are (means + SE) for the given number of rats. Analysis of variance (ANOVA) with repeated measures (BMD P2V) (Dixon and Brown, 1979) and ANOVA with Dunnett's test were used for statistical analysis of the data.

Drugs used. (±)Anatoxin-A was synthesized by Dr. Rick L. Danheiser (MIT) (Danheiser *et al.*, 1985). The stock solution dissolved in ethanol was stored at −20°C under argon until used. The drug dilutions were made in physiological saline (0.9%) immediately before each injection. Nicotine (Sigma) and chlorisondamine (Ciba-Geigy) were dissolved in saline.

RESULTS

Systemic Effects of Anatoxin-A and Nicotine

Effects on blood pressure and heart rate. Anatoxin-A and nicotine were injected intravenously at doses of 10, 30, 100, and 300 μg/kg. At the two higher doses both anatoxin-A and nicotine produced dose-related increases in mean arterial pressure (Fig. 1). The effect became apparent immediately after the injection reached its maximum within 1–2 min, and completely subsided in 5–10 min. Low

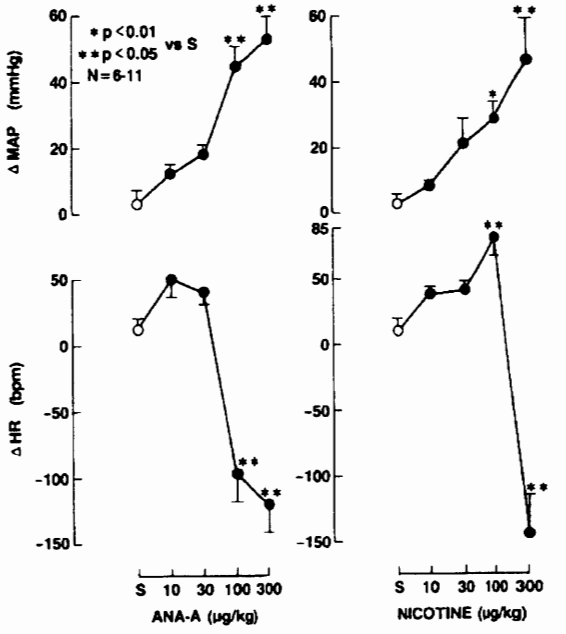

FIG. 1. Effect of intravenously administered anatoxin-A (ANA-A) and nicotine on mean arterial pressure and heart rate in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (50 μl/100 g). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (*N*) is given in the graph.

doses of anatoxin-A tended to increase heart rate while high doses elicited bradycardia (heart rate decreased more than 100 bpm). Nicotine at the 100 μg/kg dose increased heart rate while the 300 μg/kg dose reduced heart rate.

Effects on cardiac index and total peripheral resistance index (TPRI). The effect of anatoxin-A and nicotine on cardiac index and TPRI are presented in Fig. 2. At the 100 μg/kg dose anatoxin-A or nicotine had no effect on cardiac index. At the highest dose (300 μg/kg) anatoxin-A induced a transient increase in cardiac output. Nicotine at this dose first decreased (phase I) and then 5 min after the injection (phase II) increased cardiac output. The initial phase I of the nicotine effect was accompanied by an increase in TPRI but resistance remained unchanged during phase II of the nicotine response.

Effects on regional blood flow and vascular resistance. The hindquarter blood flow was

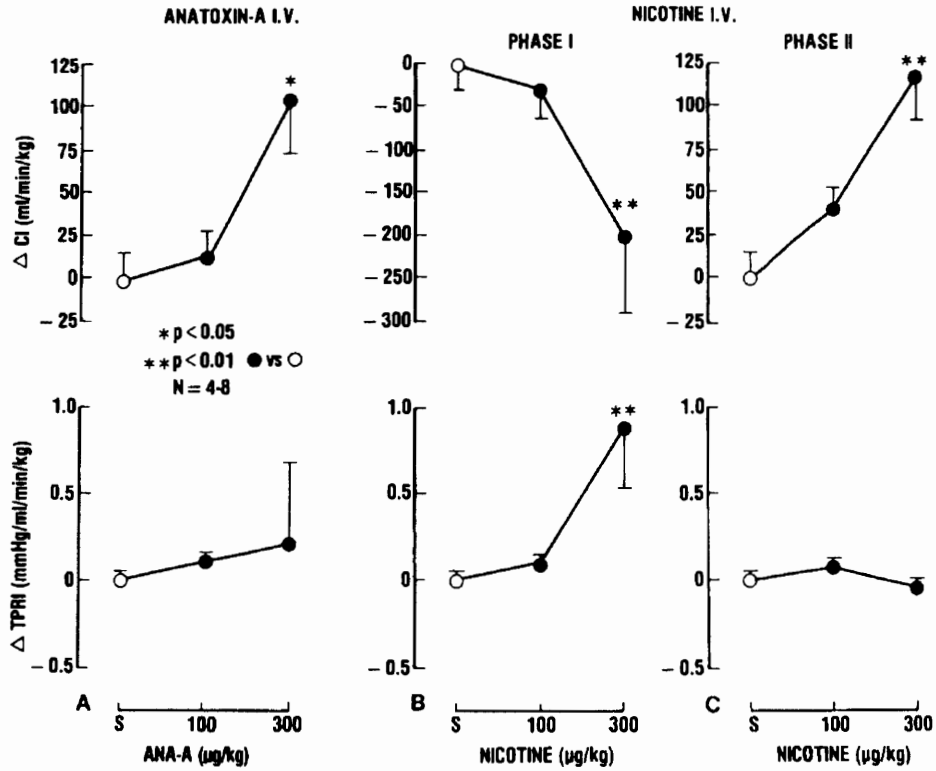

FIG. 2. Effect of intravenously administered anatoxin-A (ANA-A) and nicotine on cardiac index (CI) and total peripheral resistance index (TPRI) in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (50 μl/100 g). Significance versus saline is indicated by asterisks (Dunnett's test). (A) ANA-A effect; (B, C) the two phases of nicotine effect (each phase representing changes from the baseline immediately before nicotine administration). Number of animals (N) is given in the graph.

increased by anatoxin-A or nicotine doses of 100 and 300 μg/kg, but the vascular resistance in the hindquarter vascular bed was not changed (Fig. 3). In the renal (Fig. 4) and particularly mesenteric (Fig. 5) blood vessels both anatoxin-A and nicotine produced vasoconstriction as evidence by decreased blood flow and increased vascular resistance.

Role of the Autonomic Nervous System

The role of sympathetic nervous system in mediating cardiovascular responses to anatoxin-A was studied (1) by monitoring plasma catecholamines after anatoxin-A and nicotine administration and (2) by treating the rats with the ganglionic blocker chlorisondamine.

Effect of anatoxin-A and nicotine on plasma catecholamines. Anatoxin-A at the dose of 100 μg/kg produced a 2-fold increase in plasma epinephrine but had no significant effect on plasma norepinephrine levels (Fig. 6). The corresponding dose of nicotine increased both epinephrine and norepinephrine by 20- to 30-fold (Fig. 6).

Influence of chlorisondamine. Chlorisondamine (5 mg/kg iv) completely blocked the pressor effect of anatoxin-A (100 μg/kg) and reduced the tachycardic response (Fig. 7). The decrease in hindquarter blood flow by anatoxin-A was totally blocked after chlorisondamine while the flow in renal and mesenteric beds was still decreased by anatoxin-A administration in the chlorisondamine-treated rats (Fig. 7). The increase in vascular resistance by anatoxin-A in the all these vas-

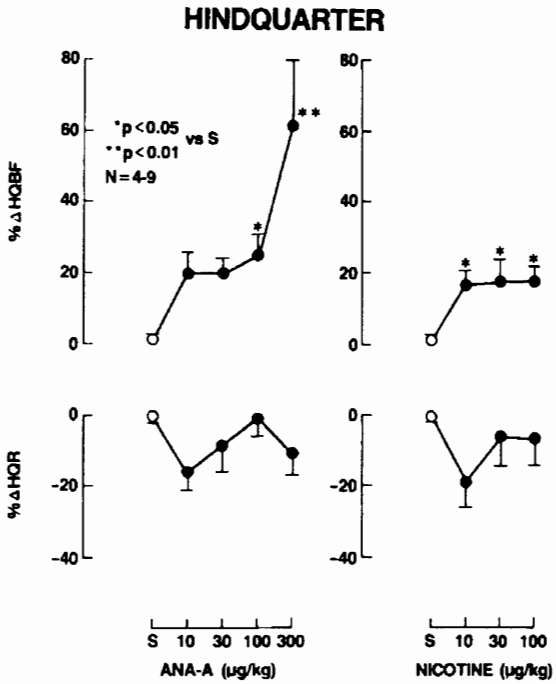

FIG. 3. Effect of intravenously administered anatoxin-A (ANA-A) and nicotine on hindquarter blood flow (HQBF) and vascular resistance (HQR) in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (50 µl/100 g). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (N) is given in the graph.

cular beds, however, was blocked by chlorisondamine.

Chlorisondamine reversed the pressor effect of nicotine (100 µg/kg) and attenuated the tachycardia produced by nicotine (Fig. 8). Nicotine decreased blood flow in hindquarter, renal, and mesenteric vascular beds in chlorisondamine-treated rats but the decrease in flow was not due to active vasoconstriction since no increase in vascular resistance was produced by nicotine after chlorisondamine treatment (Fig. 8).

Central Effects of Anatoxin-A and Nicotine

Effects on blood pressure and heart rate. Intracerebroventricular administration of anatoxin-A at the dose of 100 µg/kg increased blood pressure (Fig. 9). Nicotine at doses of 30 and 100 µg/kg induced a dose-related hypertensive response (Fig. 9). The heart rate was not significantly altered by icv-administered anatoxin-A or nicotine.

Effects on regional blood flow and vascular resistance. The blood flow and vascular resistance in hindquarters did not significantly change after icv administration of anatoxin-A or nicotine (Fig. 10). Renal blood flow was decreased and the renal vascular resistance increased after the icv doses of 10 and 30 µg/kg of anatoxin-A and of 10 and 100 µg/kg of nicotine (Fig. 10). The decrease in renal blood flow after the 30 µg/kg dose of nicotine (not displayed in Fig. 10) was −32 ± 10% (n = 2). The corresponding increase in renal resistance at this dose was +71 ± 44% (n = 2). The mesenteric blood flow significantly de-

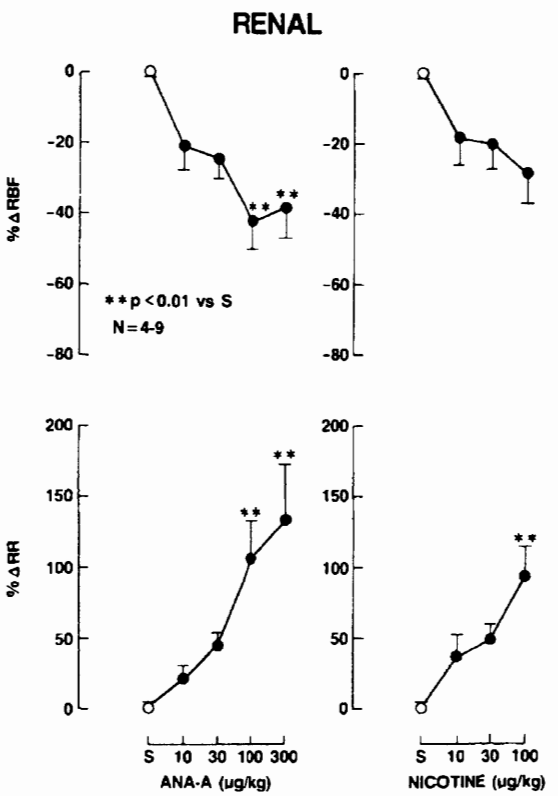

FIG. 4. Effect of intravenously administered anatoxin-A (ANA-A) and nicotine on renal blood flow (RBF) and vascular resistance (RR) in the conscious rat. The values (means + SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (50 µl/100 g). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (N) is given in the graph.

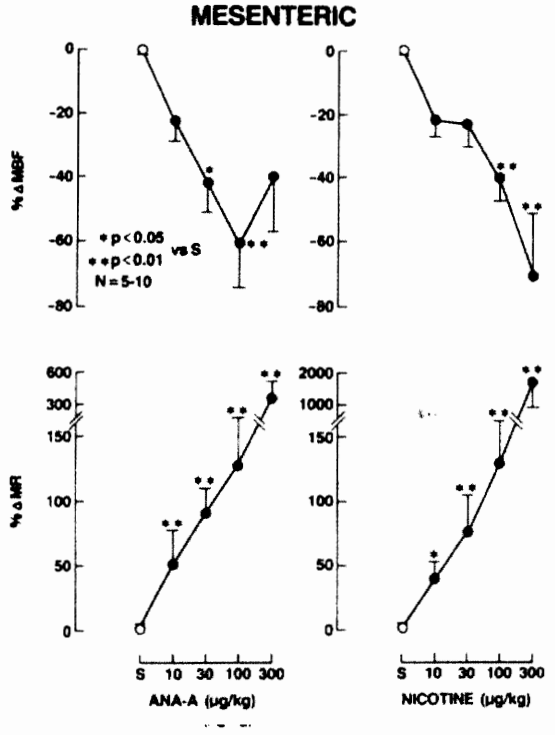

FIG. 5. Effect of intravenously administered anatoxin-A (ANA-A) and nicotine on mesenteric blood flow (MBF) and vascular resistance (MR) in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (50 μl/100 g). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (N) is given in the graph.

creased and the mesenteric vascular resistance increased after the 100 μg/kg dose of anatoxin-A and the 30 and 100 μg/kg doses of nicotine (Fig. 10).

DISCUSSION

The present results demonstrate that anatoxin-A and nicotine produce potent cardiovascular changes after both systemic and central nervous system administration. Intravenous administration of anatoxin-A or nicotine (100–300 μg/kg) induced a pressor response (Fig. 1). Vasoconstriction in renal and mesenteric circulation most likely contributed to the increase in blood pressure since the vascular resistance in these blood vessels was significantly increased at the peak of the pressor response (Figs. 3–5). The potency of the constrictor response was more than threefold greater in the mesenteric (Fig. 5) than in the renal (Fig. 4) blood vessels indicating most probably the capacity of the renal vasculature to autoregulate its blood flow.

At the highest doses of anatoxin-A and nicotine an increase in cardiac output also contributed to the pressor effect (Fig. 2). Nicotine but not anatoxin-A induced a biphasic change in cardiac output; preceding the increase, cardiac output was transiently decreased immediately after nicotine administration. The depressant effect could be due to vagal stimulation of the heart since it was also accompanied by bradycardia (Fig. 1). However, increased cardiac afterload due to pe-

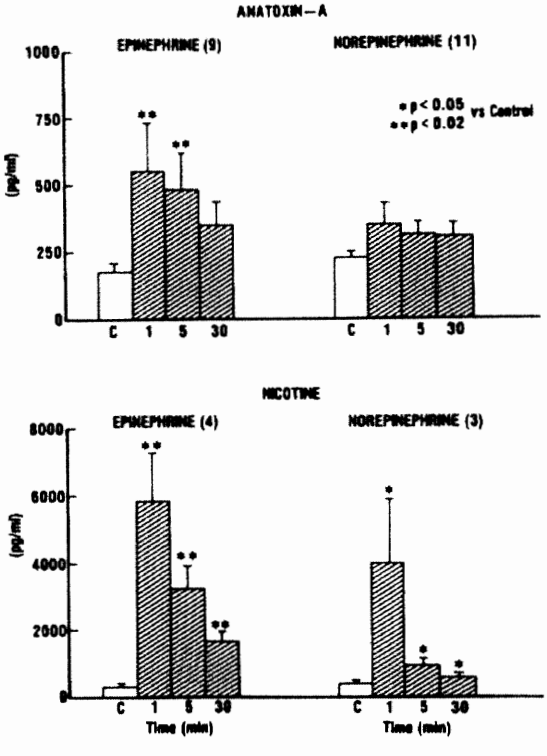

FIG. 6. Effect of intravenously administered anatoxin-A and nicotine on plasma catecholamine levels in the conscious rat. The values (means ± SEM) indicate levels of plasma epinephrine and norepinephrine at control (C) and 1, 5, and 30 min after administration of anatoxin-A or nicotine. Statistical significance is indicated by asterisks (Dunnett's test). Numbers of animals are given in parentheses.

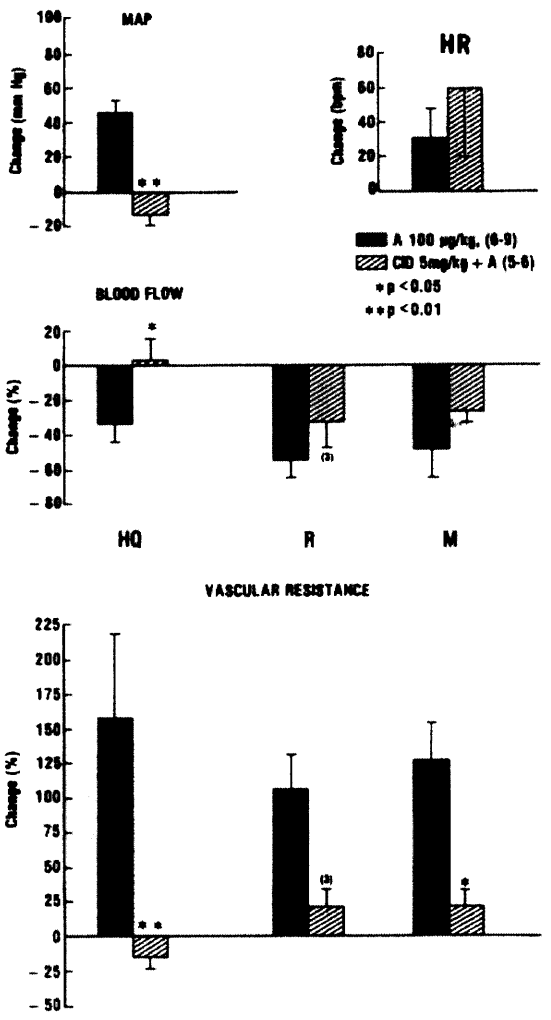

FIG. 7. Influence of chlorisondamine (CID) on the cardiovascular effects of intravenously administered anatoxin-A (A) in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before anatoxin-A administration. Chlorisondamine (5 mg/kg) was injected iv 20 min before administration of anatoxin-A at a dose of 100 µg/kg. Statistical significance is indicated by asterisks (Dunnett's test). Numbers of animals are given in parentheses. MAP: mean arterial pressure; HR: heart rate; HQ: hindquarter; R: renal; M: mesenteric.

ripheral vasoconstriction also contributed to the reduced cardiac output since total peripheral resistance was significantly increased at the nadir of the cardiac depressant effect. Interestingly, anatoxin-A only increased cardiac output despite its bradycardic response. The increase in cardiac output, however, preceded the bradycardic response which probably was due to reflex vagal activation in response to peripheral vasoconstriction (Brezenoff and Giuliano, 1982).

The hemodynamic effects of intravenously administered anatoxin-A and nicotine were effectively blocked by the ganglion blocker chlorisondamine (Figs. 7 and 8) indicating that these effects were mediated by the nicotinic receptors in autonomic ganglia. Thus, our results corroborate the *in vitro* data that anatoxin-A is a potent nicotinic cholinergic agonist (Carmichael *et al.*, 1979; Swanson *et al.*, 1986; Zhang *et al.*, 1987). Activation of adrenal medulla was also evident by the increased levels of plasma epinephrine after anatoxin-A and nicotine administration (Fig.

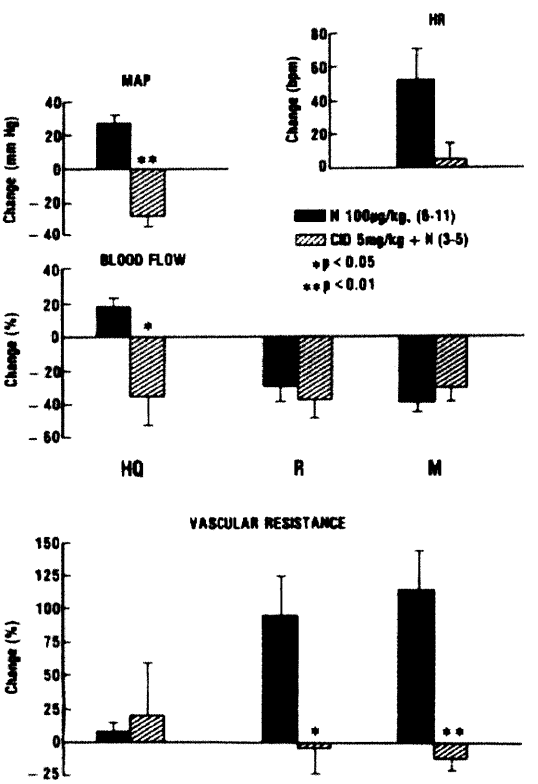

FIG. 8. Influence of chlorisondamine (CID) on the cardiovascular effects of intravenously administered nicotine (N) in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before nicotine administration. Chlorisondamine (5 mg/kg) was injected iv 20 min before administration of nicotine at a dose of 100 µg/kg. Statistical significance is indicated by asterisks (Dunnett's test). Numbers of animals are given in parentheses. MAP: mean arterial pressure; HR: heart rate; HQ: hindquarter; R: renal; M: mesenteric.

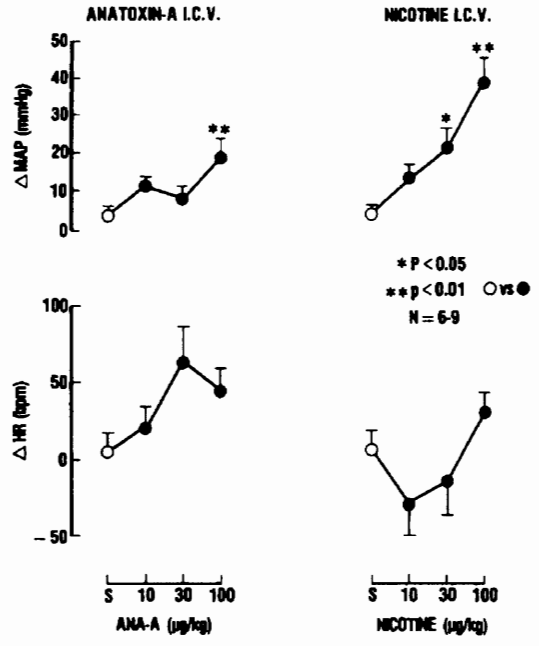

FIG. 9. Effect of intracerebroventricularly administered anatoxin-A (ANA-A) and nicotine on mean arterial pressure and heart rate in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (10 μl). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (*N*) is given in the graph.

6). Paradoxically, we found a striking difference in the potency of anatoxin-A versus nicotine to increase plasma catecholamine levels: nicotine was more than 10-fold more potent than anatoxin-A in provoking catecholamine release; yet anatoxin-A seemed to stimulate selectively the adrenal medulla with virtually no effect on the sympathetic nerves since only plasma epinephrine but not norepinephrine levels were significantly elevated after anatoxin-A administration. Nicotine, on the other hand, increased both plasma epinephrine and norepinephrine. Since in the rat 30–45% of the circulating norepinephrine is estimated to be derived from the adrenal medulla (Goldstein *et al.*, 1983; Sirén *et al.*, 1988), the increase in plasma norepinephrine after nicotine is likely to be due to an activation of the sympathetic nerves and adrenal medulla. Stimulation of the sympathetic nerves (as evidenced by the increase in plasma norepinephrine level) probably also contributed to the strong vasoconstrictor effect and increased TPR seen immediately after nicotine but not anatoxin-A administration. These findings concur with the earlier studies which demonstrated dissociation of the pressor and adrenal catecholamine releasing effects of cholinomimetic drugs (see Brezenoff and Giuliano, 1982; Kaul and Grewal, 1972; Brezenoff, 1973). Nonetheless, a role for adrenal catecholamines in mediating the hemodynamic responses to anatoxin-A cannot be excluded. Epinephrine released from adrenal medulla probably produced both vasodilator and vasoconstrictor responses (Sirén *et al.*, 1988) which could account for the lack of an initial vasoconstrictor response after anatoxin-A administration (Fig. 2). Interestingly, the anatoxin-A dose of 100 μg/kg produced only reflex bradycardia and not tachycardia like the corresponding dose of nicotine (Fig. 1). Since tachycardia was shown to reflect reliably the alterations in sympathetic tone in situations in which the sympathoadrenomedullary system has been stimulated (Ricksten *et al.*, 1984), the lack of a tachycardic response after anatoxin-A administration further indicated a major role for the adrenal medulla rather than sympathetic nerves in mediation of the anatoxin-A effects. These findings thus imply that despite the critical role of ganglionic nicotine receptors in mediating the hemodynamic effects of anatoxin-A and nicotine, the mechanisms involved in the cardiovascular actions of these substances may not be entirely identical.

Intracerebroventricular administration of anatoxin-A and nicotine (30–100 μg/kg) induced pressor and splanchnic vasoconstrictor responses (Figs. 9 and 10). Again, the mesenteric vasculature was the most sensitive to the constrictor action of anatoxin-A and nicotine (Fig. 10). Our results thus agree with and extend the previous finding that icv-administered acetylcholine or nicotine in the rat increase blood pressure with variable effect on the heart rate (see Brezenoff and Giuliano,

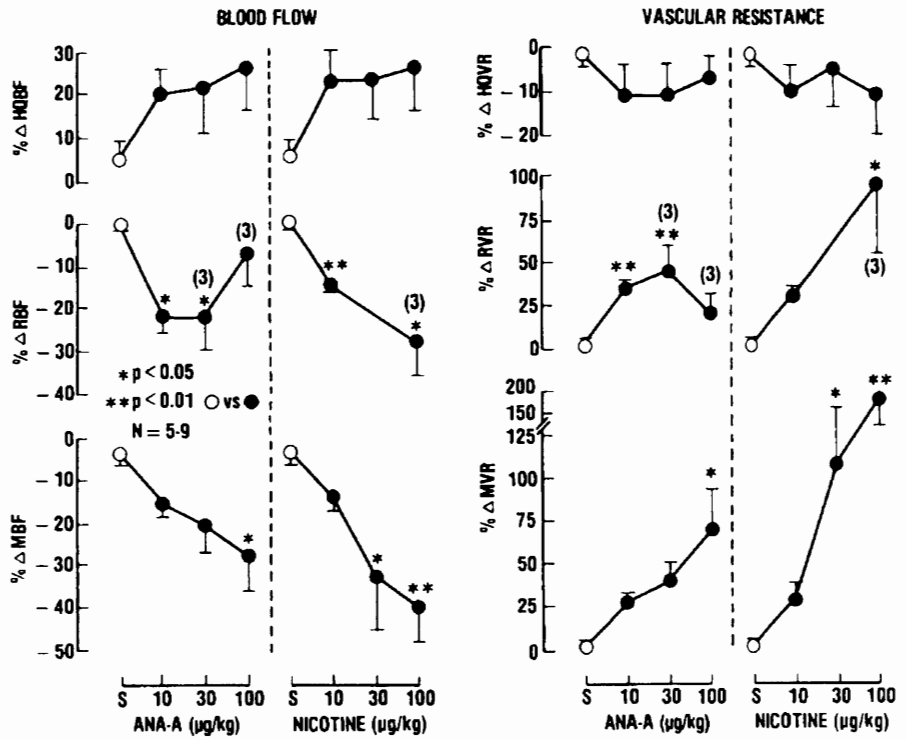

FIG. 10. Effect of intracerebroventricularly administered anatoxin-A (ANA-A) and nicotine on regional blood flow and vascular resistance in the conscious rat. The values (means ± SEM) indicate maximum changes from the baseline before ANA-A or nicotine administration. Open circles denote the effect of saline (10 μl). Significance versus saline is indicated by asterisks (Dunnett's test). Number of animals (*N*) is given in the graph. HQ: hindquarter; R: renal; M: mesenteric; BF: blood flow; VR: vascular resistance.

1982). In the anesthetized rat, intracisternal or icv administration of nicotine was reported to increase blood pressure (Kubo and Misu, 1980; Krstic and Djurkovic, 1978). The cardiovascular effects of anatoxin-A in our study most likely were due to stimulation of cholinergic nicotinic receptors since anatoxin-A was shown to bind to nicotine binding sites in the rat brain (Zhang *et al.*, 1987). The weaker potency of anatoxin-A as compared to nicotine to produce cardiovascular responses after icv administration might be due to the use of the racemic mixture of (+)- and (−)-isomers of anatoxin-A rather than the pure (+)-isomer since in the rat brain the (+)-isomer of anatoxin-A was reported to readily displace nicotine from its high-affinity binding sites whereas the (−)-isomer only bound to the low-affinity site (Zhang *et al.*, 1987).

In summary, the present study demonstrates that the cardiovascular responses to anatoxin-A bear close resemblance to those of a nicotinic cholinergic agonist after both systemic and central nervous system administration in the conscious rat. Anatoxin-A and nicotine produced pressor and renosplanchnic vasoconstrictor responses and at high doses increased cardiac output. These effects were most probably mediated by activation of the nicotinic receptors in sympathetic ganglia and adrenal medulla. Interestingly, marked differences were found in the ability of anatoxin-A versus nicotine to stimulate the sympathoadrenomedullary axis. Since both nicotine and anatoxin-A bind preferentially to nicotinic cholinergic binding sites our data might imply that the nicotinic receptors in the adrenal medulla are disparate from the nicotine receptors in sympathetic ganglia.

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G39231. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or as necessarily reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*. Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 85-23, 1985). The authors thank Mrs. B. Vanatta for excellent technical assistance.

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