

Effects of Nicotine on the Subcutaneous Microcirculation of the Bat^{1,2} (34429)

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(Introduced by R. N. Harger)

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The widespread consumption of nicotine contained in tobacco smoke and the correlation of smoking with adverse effects on vascular diseases (1) have resulted in many investigations of the cardiovascular effects of nicotine. These were recently summarized by Whelan (2). Roth *et al.* (3) administered 2 mg of nicotine intravenously to humans and observed an increase in blood pressure and heart rate. Roth and Shik (4) found that skin vessel constriction produced by cigarette smoking was abolished by sympathectomy of the extremity and they postulated that this effect was due to nervous reflex activity. Fewings *et al.* (5) utilized occlusive venous plethysmography in human subjects to show that intra-arterial injection of nicotine produced a profound and persistent vasodilation in both skin and skeletal muscle. Albertini *et al.* (6) reported an initial constriction followed by a profound vasodilation in the rat mesentery for intravenous administration of 0.5 mg/kg of nicotine.

These various studies present conflicting data for the effects of nicotine upon the peripheral vasculature. Some conflict may arise from comparison of observations on unanesthetized humans to those of anesthetized animals. In addition, contradictions may result because interpretations of micro-

circulatory responses were based upon the *indirect* measurement of microcirculatory variables. The present communication reports the effects of intraperitoneal administration of nicotine on subcutaneous minute arteries and veins which were *directly* observed in the wing of the unanesthetized bat.

Materials and Methods. To allow selection of an appropriate nicotine dose for the vascular studies, the toxicity of nicotine was determined in the bat (*Myotis lucifugus*). Nicotine in normal saline was administered intraperitoneally to bats at five dose levels with 24.1 mg/kg of body weight as the lowest dose. Each of the higher doses was taken as 1.2 times the next lower dose. With equal numbers of males and females at each dose level, the LD₅₀ value for a 24-hr period was determined by the method of Weil (7).

Vascular studies were conducted on the bat preparation described by Nicoll and Webb (8) which was recently modified by Harris *et al.* (9). An unanesthetized bat was placed on his back with both wings held in an extended position over a glass plate by spring clips. The plate was placed on the stage of a binocular microscope and a microscopic field was illuminated by a 300-W zirconium arc lamp. The light was collected by an objective lens (10×) and divided into two components by a beam splitter. Approximately 90% of the collected light passed through a focusing lens (10×) to an Ampex television camera. The resulting video signal was presented for analysis as a picture at 1000× total magnification on an Ampex television monitor.

Minute artery (approx 30 μ) and vein (approximately 70 μ) diameters were determined at 30-sec intervals by marking and measur-

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ing the vessel walls on a previously calibrated television monitor screen. Minute vein vasomotion (the number of contractions per minute) was counted for successive 1-min intervals. Electrodes on the legs (held in place with collodion) provided an ECG signal which was amplified and recorded on a Sanborn Polygraph. Heart rate was calculated from 6-sec recordings which were taken every 5 min. Rectal temperature was measured with a bead thermistor, while room temperature was kept between 25–30°.

The experimental protocol for each experiment consisted of a 10-min control period, an intraperitoneal injection of nicotine in saline (or a saline blank), and a 50-min postinjection observational period. All solutions, including those used in the LD₅₀ study, were prepared in appropriate concentrations so that a standardized volume of 10 μ liters/g of body weight was injected.

The animals were divided into four experimental groups, with one experiment performed on each animal. The first group (6 animals) with an intact sympathetic nervous system received a saline blank injection. The second group (4 animals) also received a saline blank injection; however, the observed vessels had undergone sympathetic denervation 6 or 7 days prior to data collection. Denervation was accomplished under local procaine anesthesia in the axillary region by surgically removing a 2–3 mm section of the tregmental nerve. In all denervation studies, vessels with approximately the same anatomical location in the wing as those used in the innervation studies were selected for observation. The third group (4 animals) with an intact sympathetic nervous system was injected intraperitoneally with a nicotine-saline solution at a dose of 20 mg of nicotine/kg of body weight. The fourth experimental group (4 animals) with sympathetic vascular denervation, as described for group two, also received 20 mg of nicotine/kg of body weight intraperitoneally. The 20 mg/kg dose was selected since the toxicity study indicated that this was approximately one half the LD₅₀ value.

Raw data for each variable in each experiment were "smoothed" and "normalized."

"Smoothing" was accomplished by a five-point digital computer filter in which each raw data value, $X(N)$, was converted to a smoothed data value $X_s(N)$, by the following formula:

$$X_s(N) = \frac{X(N-2)}{16} + \frac{X(N-1)}{4} + \frac{3X(N)}{8} + \frac{X(N+1)}{4} + \frac{X(N+2)}{16}$$

As an example for minute artery diameter, which was measured at 0.5-min intervals, the smoothed value at 30 min was calculated as:

$$X_s(\text{at } 30 \text{ min}) = \frac{X(29 \text{ min})}{16} + \frac{X(29.5 \text{ min})}{4} + \frac{3X(30 \text{ min})}{8} + \frac{X(30.5 \text{ min})}{4} + \frac{X(31 \text{ min})}{16}$$

Smoothing data in this manner reduces random errors which may be introduced by sampling a variable which is changing continuously. "Normalization" was achieved for each variable by calculation of the average value during the 10-min control period for each experiment. All data for each variable in each experiment was then divided by this average control value to convert the smoothed data to percentages. The normalized data for each variable in all experiments were averaged at each point in time to obtain the data which are presented graphically in this communication.

Results. The LD₅₀ for the mixed population (raw data presented in Table I) was 39.7 mg/kg of body weight with 95% confidence limits of 33.1–47.7 mg/kg. At dose levels well below the LD₅₀, nicotine appeared to depress the normal activity of the bat.

TABLE I. Summary of Acute Intraperitoneal Toxicity (24 hr) of Nicotine in Bats.

Doses (mg/kg)	Deaths
50	5/6
41.6	3/6
34.8	2/6
28.9	1/6
24.1	0/6

Climbing ability was impaired with the animals remaining quiet on the floor of the cage. Even at low doses, ability to fly was impaired. Administration of nicotine at a dose near or above the LD_{50} dose resulted in marked stimulation. Within several minutes, the bat was tremulous with an apparent increase in respiration. In most instances, death appeared to follow a convulsion during which the bat became rigid with hind leg extension.

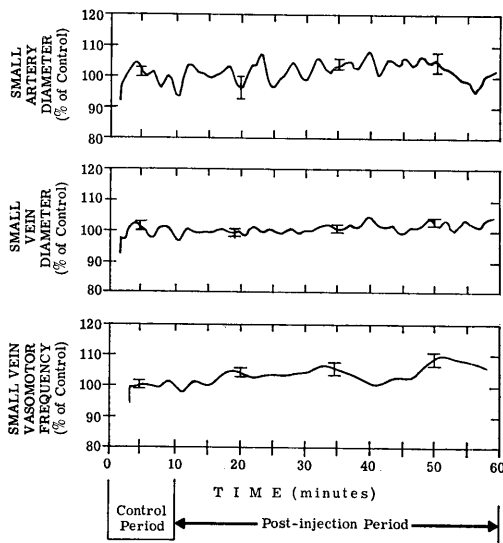


FIG. 1. Mean responses of minute blood vessels for 6 bats with intact wing innervation which were injected with normal saline. The abscissa is time in minutes with 0 and 10 min corresponding to the start of the control and postinjection periods, respectively. Each ordinate (small artery diameter, small vein diameter, and small vein vasomotor frequency) is expressed as a percentage of the control period. Vertical lines represent standard errors at 5, 20, 35, and 50 min.

The average vascular responses are presented in Fig. 1-4 as previously described under "Methods." The times 5, 20, 35, and 50 min were arbitrarily selected for calculation of standard errors.

Figures 1 and 2 give data for group 1 (innervated vessels receiving saline) and group 2 (denervated vessels receiving saline) respectively. Neither group showed significant changes in minute artery and vein diameters nor in minute vein vasomotion.

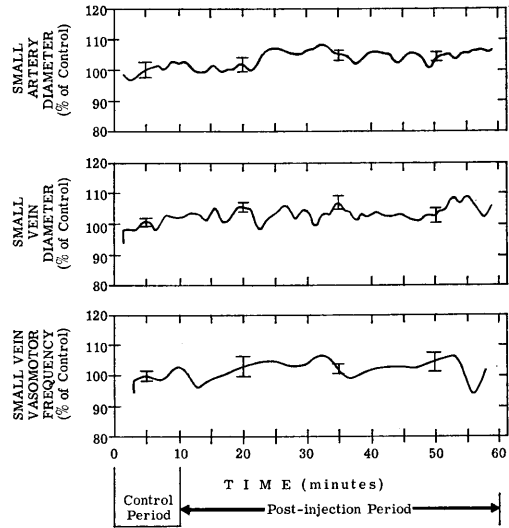


FIG. 2. Mean responses of minute blood vessels for 4 bats with prior sympathetic denervation which were injected with normal saline. The ordinate, abscissa, and vertical lines are the same as those for Fig. 1.

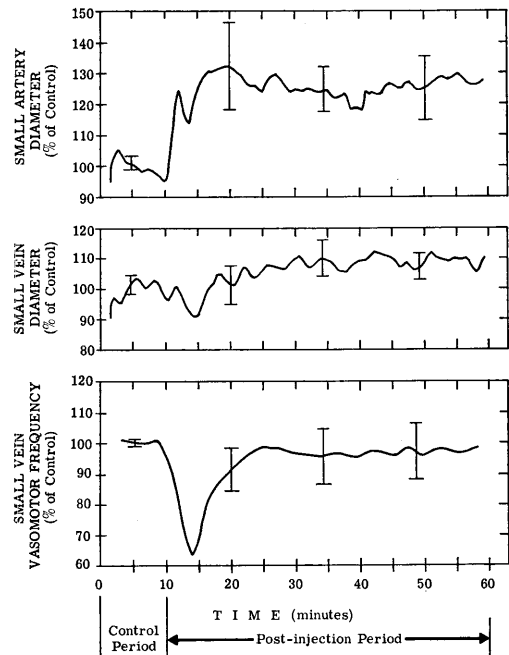


FIG. 3. Mean responses of minute blood vessels for 4 bats with intact sympathetic wing innervation which were administered nicotine intraperitoneally (20 mg/kg of body wt) at the 10-min mark. The ordinate, abscissa, and vertical lines are the same as those in Fig. 1.

TABLE II. Heart Rate Data for 8 Bats Treated with Nicotine (ip) Compared to Averaged Data for 8 Bats Treated with Saline (ip).

Av heart rate ^a during 10-min control	Percentage of av control heart rate												
	0	5	10	15	20	25	30	35	40	45	50	55	60
282 ± 25 ^b	108 ± 4	98 ± 4	93 ± 2	95 ± 5	96 ± 7	92 ± 7	98 ± 8	95 ± 6	96 ± 7	95 ± 8	92 ± 8	90 ± 9	94 ± 11
313 ^c	115	92	92	92	166	191	207	201	194	191	188	182	182
230	86	108	104	252	252	226	221	221	217	213	204	204	204
327	110	97	91	204	195	192	201	204	207	201	186	189	189
470	87	104	108	140	127	119	114	112	112	114	114	114	117
640	98	98	103	87	78	79	92	93	95	95	87	81	81
350	108	91	100	131	114	97	91	85	82	80	91	77	94
417	110	95	93	136	124	117	112	110	110	110	110	112	115
580	100	103	96	100	87	84	81	79	79	72	74	74	74
416 ± 50 ^d	102 ± 4	98 ± 2	98 ± 2	143 ± 21	143 ± 21	138 ± 20	140 ± 20	138 ± 21	137 ± 20	134 ± 21	132 ± 18	129 ± 19	132 ± 19

^a Average of heart rate at 0, 5, and 10 min.^b Average heart rate with standard errors for saline-treated animals.^c Each row is data for one nicotine experiment.^d Average heart rate with standard errors for nicotine-treated animals.

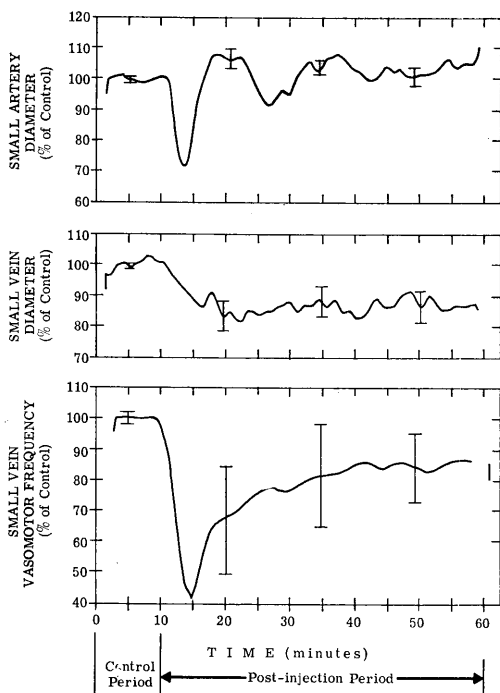


FIG. 4. Mean responses of minute blood vessels for 4 bats with prior sympathetic denervation which were administered nicotine intraperitoneally (20 mg/kg of body wt) at the 10-min mark. The ordinate, abscissa, and vertical lines are the same as those in Fig. 1.

Figure 3 represents the averaged responses for group 3 (innervated vessels receiving nicotine). Within 5 min after nicotine injection, minute artery diameter increased approximately 30% above control level and remained dilated throughout the experimental period. Minute vein diameter increased about 10% for the last 40 min of the experimental period. Minute vein vasomotion sharply decreased about 40% immediately upon injection with nicotine, but after 25 min it almost returned to the control level.

Data for group 4 (denervated vessels receiving nicotine) are shown in Fig. 4. In contrast to that for the innervated series, minute artery diameter decreased 30% 3–4 min after injection and then 10 min later it tended to fluctuate about the mean control diameter. Minute vein diameter decreased about 15% with 5% fluctuations around this value. Within 5 min following nicotine injection,

minute vein vasomotion decreased approximately 60% and then slowly returned to approximately 80% of the control value within 20 min after injection. Vasomotion remained at this level for the remaining 30 min of the experimental period.

Table II contains normalized heart rate data for the control (Groups 1 and 2) and nicotine (Groups 3 and 4) experiments. In the control experiments heart rate demonstrated variability but there was no systematic change over the entire protocol period. A tachycardia occurred immediately after the injection of nicotine and it persisted throughout the experiment. A paired *t* test was not significant for the change in heart rate at 15, 35, 60 min in comparison to the end of the control period. However, three of the eight animals had an average control heart rate of 470, 580, and 640 beats/min which was greater than the 95% confidence limits for resting heart rate in the bat (282 ± 155). During the control period, these three animals appeared to be in a state of tachycardia and did not give an exaggerated tachycardia after administration of nicotine.

Discussion. In Table III, the intraperitoneal LD_{50} of nicotine in bats is compared to those reported for other species. The LD_{50} of nicotine in the bat is higher than that reported for the mouse, rat, and rabbit.

Orcutt *et al.* (12) described eight stages of the central nervous system effects of nicotine when administered intravenously to the rat: 1. prostration; 2. fine tremor; 3. clonus; 4. clonic convulsions; 5. opisthotonos; 6. tonic flexion; 7. tonic extension; and 8. death (by respiratory paralysis). In general our observations of the effects of nicotine on the bat are consistent with several of the stages described by Orcutt. Because of the wide varia-

TABLE III. Intraperitoneal Toxicity of Nicotine to Various Species.

Species	LD_{50} (mg/kg)	Ref.
Bat	39.7 ^a	Present study
Mouse	10.3	(10)
Rat	30	(11)
Rabbit	14	(10)

^a 33.1–47.7 mg/kg (95% confidence limits).

bility in toxicity and capabilities of various species to eliminate nicotine (13), the significance of the lower toxicity of nicotine to the bat is unknown. It is possible that bats have a high metabolic rate which allows very rapid elimination of nicotine and thus reduces its toxicity.

Nicotine caused a striking increase in the diameter of innervated minute arteries and a modest increase in minute vein diameter. If these responses were also present in other corresponding segments of the wing vasculature, an overall decrease in skin peripheral resistance would have occurred as expected from the indirect measurements of Fewings *et al.* (5). Fewings *et al.* (5) noted that vasodilation took place in the sympathectomized limb, and concluded that vasodilation resulted from the direct action of nicotine on the vasculature. However, our studies indicate that sympathetically denervated minute arteries responded to nicotine with a transient constriction followed by little change from control values for the remainder of the experimental period. In the innervated wings, minute veins responded to nicotine with vasodilation which accompanied a brief depression in minute vein vasomotion. In contrast to this, the minute vein response for the denervated series consisted of vasoconstriction which was also accompanied by a depression in minute vein vasomotion. These data suggest that nicotine may produce vasoconstriction by direct action on the peripheral vasculature and that minute vein dilatation for nicotine may have been mediated via the sympathetic system. However, we cannot presently exclude the possibility that a passive dilation of the minute vein occurs for the innervated series because minute artery dilatation may have increased minute vein pressure and flow. The vasomotion responses were similar in both the innervated and denervated series with an apparent enhancement of the response for sympathetic denervation, thus the vasomotion response also supports the concept of a direct action of nicotine. This concept is further supported by a series of experiments by Nicoll (14) in which he subjected bats to cigarette smoke and noted an initial increase in the contraction phase of noninner-

vated terminal arterioles. It is significant that in the denervated series, a depression in minute vein vasomotion was associated with minute vein constriction since previous investigations of vascular responses to ethanol (9) and also to hemorrhagic hypotension by Harris *et al.* (submitted for publication) have usually associated a decrease in vasomotion with an increase in minute vein diameter. Therefore, our data suggests that minute vein vasomotion can be manipulated independently of minute vein diameter.

Characteristically, nicotine given intravenously to experimental animals produces an initial bradycardia followed by tachycardia (15, 16). Similar responses have also been reported for inhalation of cigarette smoke (17). In our work, neither series of experimental animals demonstrated a decreased heart rate. It is possible that a transient bradycardia may have occurred but was missed because heart rate was only recorded at 5-min intervals.

As summarized by Larson and Silvette (18), the current concept holds that nicotine induces tachycardia by release of catecholamines from the adrenals or from sympathetic endings including those in the heart. An evident and persistent tachycardia is consistent with previous observations but does not allow conclusions about the proposed mechanism.

Summary. The intraperitoneal toxicity of nicotine was studied in bats (*Myotis lucifugus*). The LD₅₀ was 39.7 mg/kg (33.1–47.7 mg/kg; 95% confidence limits). Thus, nicotine appears to be less toxic to the bat than to the mouse, rat, or rabbit.

Direct quantitative observations of vascular diameters in unanesthetized bats were made with a microscope combined with a closed circuit television system. The diameter of innervated minute arteries markedly increased with intraperitoneal nicotine administration. Minute vein diameter slightly increased while minute vein vasomotion slightly decreased. Sympathetic denervation abolished the increase in minute artery diameter when nicotine was administered. However, minute vein diameter decreased and was also accompanied by decreased minute vein vasomotion. Nicotine caused an increase in

heart rate which was not found to be significant. These data suggest that nicotine may produce vasoconstriction by direct action on the peripheral vasculature, and that minute vein dilatation may have been mediated via the sympathetic system. However, we cannot presently exclude the possibility that a passive dilation of the minute vein occurs for the innervated series because minute artery dilatation may have increased minute vein pressure and flow. Since a decrease in vasomotion has usually been found to accompany an increase in minute vein diameter, our data suggests that minute vein diameter and vasomotion can be manipulated independently.

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