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Differential Effects of Chronic Nicotine and Tobacco Smoke Administration on Iliac Vascular Resistance in the Rat (40558)[1, 2]

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Although it has been established that acute inhalation of tobacco smoke will perturbate the cardiovascular system largely through the action of nicotine (1), there have been comparatively few studies devoted to examining the long-range effects of chronic tobacco smoke and/or nicotine consumption on the basal operating status of the cardiovascular system. What studies have been performed along these lines have examined either the total cardiovascular system (2) or single microvessels (3, 4). Comparisons of the long-range effects of tobacco smoke and nicotine on a given regional vascular bed have not been made.

In the present study the effects of chronic tobacco smoke and nicotine administration on the iliac (or lower abdominal) vasculature of the rat were compared. The iliac circulation was selected for study since previous evidence suggests that portions of it are affected by chronic tobacco smoke consumption (5–7).

Materials and methods. Modes of treatment: Separate groups of male Sprague–Dawley rats were administered either nicotine per os or tobacco smoke containing known quantities of nicotine. Since the time course of nicotine appearance in the blood is different between oral and tobacco smoke modes of consumption (8), plasma nicotine data from other species were used to estimate the amount of nicotine consumed by the treated animals. The following four groups of rats were used in these studies:

(i) A smoke-treated group (SM-T) the members of which were exposed daily to tobacco smoke in accordance to established techniques (8). The cigarettes were supplied by the University of Kentucky Tobacco and Health Research Institute. The ones used in this study were the reference type which generated an average of 2.2 mg of nicotine and 30 mg of tar in the combusted smoke (9).

The rats were exposed to smoke from four cigarettes per day delivered in a sequence of 40 puffs. Each puff was diluted to a concentration of 10% with room air, and held in an exposure chamber for 15 sec with a 45-sec recovery period between puffs. These parameters resemble human smoking profiles (10).

By measuring respiratory parameters under the smoke exposure conditions used, and by applying Isaac and Rand's (11) value for the percentage of inhaled nicotine absorbed into the blood stream, it was estimated that the rats used in these experiments were receiving about 0.5 mg nicotine/kg/day via tobacco smoke. This is equivalent to a human smoking 1.7 packages of cigarettes a day (10).

(ii) A second group termed the smoke control group (SM-C) was housed in the same quarters and placed in the smoke exposure chamber on the same schedule as the SM-T group. But during "treatment" they received puffs of room air instead of tobacco smoke.

(iii) A third group received nicotine treatment (NIC-T). Members of this group were housed in individual cages and given water *ad libitum* which contained 20 μg/ml of nicotine. Daily water consumption was measured and found to average 120 ml/kg body wt/day. This represents an oral nicotine intake of 2.4 mg/kg/day, a figure comparable to previous oral nicotine treatment studies with rats (4, 12).

From human studies in which plasma levels of nicotine obtained via tobacco smoke were compared with those obtained by oral nicotine consumption (13), and from studies which compared behavioral responses of humans to tobacco smoke and oral nicotine administration (14), it was estimated that oral

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nicotine administration of 2.4 mg/kg/day would yield nicotine consumption levels equivalent to those obtained by a human smoking between one and two packs of cigarettes a day. Thus, the levels of nicotine obtained by tobacco smoke and oral consumption in the present study should be reasonably equivalent.

Treatment durations for the groups ranged from 4 to 6 months, a time period which on the basis of previous studies would be more than sufficient to elicit cardiovascular changes in the rat (3, 4, 6, 12). The interval between the shortest and longest treatment duration represents the period during which acute experiments were being performed on the rats. Analysis of the data showed no significant time-related variations in iliac vascular resistance over the period of experimentation for any of the groups.

Experimental preparation. After anesthesia with urethane (1 g/kg) the abdomen was entered and the lower abdominal aorta was isolated just above the iliac branches. Following heparinization of the animal the isolated aorta was cannulated in a cephalic direction with a 2-mm i.d. section of tygon tubing tapered at the end to fit in the vessel. This circuit directed blood first past a side port coupled to a Statham pressure transducer for measuring arterial perfusion pressure (P_a), then through an electromagnetic flow probe (Carolina EP606), and then back into the lower abdominal aorta for perfusion of the iliac arteries. A clamp placed proximal to the pressure port was used to vary P_a. The flow probe was coupled to a Carolina Model 322 electromagnetic flow meter for measurement of iliac blood flow (F_I). Both P_a and F_I were continuously recorded on a Grass Model 7 polygraph. After placement of the extracorporeal circuit the abdomen was closed over the tubing, and the animal's body temperature was maintained via a heat lamp placed above the preparation.

At this point the arterial tubing was momentarily occluded in order to set the zero base line of the flow meter. During this procedure mean arterial perfusion pressure ($\bar{P}_a$) decreased to an average of 12.1 mm Hg ± 3.5 SEM for all groups combined and there were no significant between-group differences. Such a low level of $\bar{P}_a$ upon occlusion of the iliac supply indicates that perfusion via collateral vessels was not significant.

Upon completion of the preparation P_a and F_I were monitored until stabilization occurred, usually within 15 to 30 min. If F_I failed to stabilize and/or if mean arterial pressure ($\bar{P}_a$) dropped below 75 mm Hg the data were not used. The number of preparations in each group which gave usable information were as follows: SM-T, n = 15; SM-C, n = 14; NIC-T, n = 16; and NIC-C, n = 13.

Experimental protocol. After recording a few minutes of steady state data, $\bar{P}_a$ was reduced by about 30 mm Hg. The preparation was kept at this lower pressure level for about 2 to 3 min following which the clamp was released and P_a returned to normal operating levels. Upon recovery from this procedure the perfusion tubing was totally occluded for a period of 3 min. The clamp was then released and the reactive hyperemic response followed. With the data generated from this protocol between-group differences in iliac vascular resistance ($R_I = \bar{P}_a/F_I$) were compared at two different levels of perfusion pressure and during maximal reactive hyperemia.

At the end of an experiment an animal was terminated by an intravenous injection of saturated KCl. The lower quarters were then removed at the level of the iliac crest and weighed. It was found that approximately 87% of the lower quarter was composed of bone, skeletal muscle, and attached skin. The remaining 13% consisted of the tail, bladder, genitalia, etc. The relative proportions of skin, muscle, and bone were not determined. This has been done in a previous study with rat hind limbs (6) in which it was found that the percentages of skin, muscle, and bone were 12, 68, and 20%, respectively, in chronic tobacco smoke-treated rats. Corresponding figures for smoke control rats were 13, 66, and 21%. These percentages are similar to those reported for the dog hind limb (15).

Results. Body and tissue weights. Table I gives weights for the various treatment groups. Note that both total body and lower quarter weights of the NIC-T and SM-T groups were lower than corresponding values for their respective control groups. Differences between corresponding treatment and control groups were significant at the $P \leq 0.05$ level or lower. These results are consist-

TABLE I. TOTAL BODY AND LOWER-QUARTER WEIGHTS

Treatment group	Total body weight (g)	Lower-quarter weight (g)
NIC-C	563.7 ± 11.8[a]	218.0 ± 5.5
NIC-T	524.9 ± 12.5	198.9 ± 5.0
SM-C	546.0 ± 17.4	236.0 ± 9.6
SM-T	497.0 ± 10.0	183.0 ± 5.3

[a] Mean ± SEM.

ent with previous observations that chronic nicotine (2, 4) and tobacco smoke (3) treatments reduce the rate of weight gain in laboratory animals.

Relationship between lower quarter tissue weight and R_I. In view of the fact that tissue weights of the treatment groups were lower than corresponding weights of the control groups (Table I), the validity of using weight as a normalizing factor in the analysis of vascular resistance was tested. Iliac resistances at normotensive perfusion pressures were calculated in units of mm Hg/ml × min, then correlated with the corresponding lower quarter weight. The results of this analysis, presented in Fig. 1, show rather dramatically that for the present study iliac vascular resistance is independent of the weight of the tissue being perfused. Therefore, unless otherwise stated, all R_I values are expressed in units of mm Hg/ml × min.

Relationship between $\bar{P}_a$ and R_I. Figure 2 presents iliac vascular resistance (R_I) as a function of mean arterial perfusion pressure $\bar{P}_a$ for the various groups. Note that for all groups, when $\bar{P}_a$ was reduced from around 90 to around 60 mm Hg, R_I increased in a manner indicative of a nonautoregulating vascular bed. Correlation analysis of these data showed that the relationship between $\bar{P}_a$ and R_I was not significantly different between corresponding treatment and control groups. The consistent lack of autoregulation in these preparations may have been a species-related phenomenon since at driving pressures below 95 mm Hg autoregulation of renal blood flow in the rat is quite modest when compared to other species (16).

With regard to the effects of nicotine and

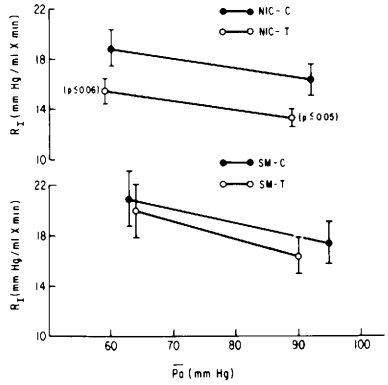

FIG. 2. Iliac vascular resistance at two levels of arterial pressure for each group. Data presented as means ± SEM. *P* values are those for significance using an independent *t* test of corresponding control vs treatment groups.

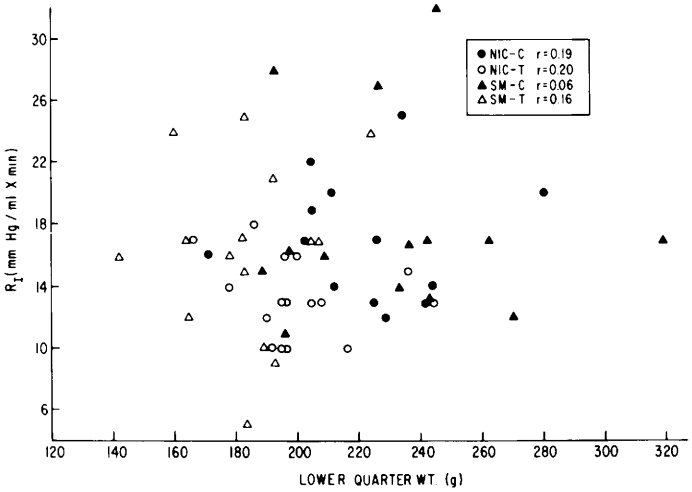

FIG. 1. Iliac vascular resistance (R_I) as a function of lower-quarter weight for animals of the various groups. The *r* values presented are the Pearson correlation coefficients for each group.

tobacco smoke treatment on absolute values of R_I, note that at each $\bar{P}_a$ level examined resistance of the NIC-T group was significantly lower than values observed in the corresponding control group. Whereas, with tobacco smoke treatment there was a tendency toward reduced vascular resistance, but differences between the SM-C and SM-T groups were not significant. Differences between the two control groups were also not significant.

Iliac resistance during reactive hyperemia. There are two general types of effects which could account for the difference in R_I observed between corresponding control and treatment groups (Fig. 2): (i) a reduction of steady-state vascular tone and/or (ii) a change in the underlying vascular architecture in a direction which favors lower resistance. The term "vascular architecture" as used in this context would include the blood vessels in their maximally dilated state, the nature of their path, the number of vessels, and the geometry of the vascular branchings.

If vascular architecture plays a significant role in the lower R_I of the NIC-T group, then relative differences in R_I between respective control and treatment groups should be the same during maximal vasodilation of the iliac vasculature, a condition which would unmask the contribution of vascular architecture to blood flow resistance. In this regard Fig. 3 presents R_I values observed at the peak of reactive hyperemia following 3 min of arterial occlusion, a procedure which elicits near maximal vasodilation. Note that R_I of the NIC-T group was significantly lower as compared to its corresponding control group. Furthermore relative differences between control and treatment groups were essentially the same during reactive hyperemia as during normal steady-state conditions. This is evidenced by the fact that the ratio of R_I for the NIC-T group to R_I for the NIC-C group was 0.79 during reactive hyperemia (Fig. 3) and 0.82 under steady-state conditions at a pressure of around 90 mm Hg (Fig. 2). In contrast to nicotine treatment, reactive hyperemia unmasked a tendency toward an increase in vascular resistance in the SM-T group but the effect was not significant (Fig. 3).

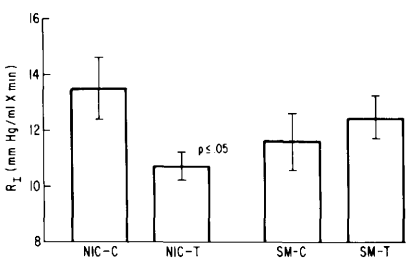

FIG. 3. Minimal iliac vascular resistances during reactive hyperemia following 3 min of arterial occlusion. Data presented as mean ± SEM for each group.

Discussion. In comparing vascular resistances at different levels of arterial pressure (Fig. 2) it is apparent that the effects of chronic tobacco smoke and chronic nicotine administration on iliac resistance were not at all similar. For while nicotine treatment did elicit a substantial decrease in iliac vascular resistance, tobacco smoke treatment had no statistically significant effect. These results are in contrast to the effects of chronic nicotine and tobacco smoke consumption in the dog where both treatments depress cardiac function and elevate total systemic resistance (2). This does not mean that the effects seen in the dog are incompatible with those of the present study. In the latter only the iliac portion of the systemic circulation was examined. Since the observed values of iliac vascular resistance in all groups (Fig. 2) are on the order of 10 times the rat's normal total peripheral resistance (17) it is unlikely that the between-group differences in R_I (Fig. 2) would be reflected at the level of the total circulation. Therefore, both nicotine and tobacco smoke treatment in the present study could have elevated resistance elsewhere in the circulation and not have modified effects on the iliac vasculature. That this might have happened is suggested from studies in which both chronic tobacco smoke (3) and nicotine (4) treatment to rats reduced capillary blood flow in the mesentery.

The tendency for nicotine consumption to reduce iliac vascular resistance vis-à-vis evidence that it has opposite effects on the circulation as a whole (2) suggests that the iliac vasculature might be quite unique in its long-term adaptation to this amine. With regard to a possible mechanism of such adaptation, Wenzel and his group (13, 18) have demonstrated that chronic nicotine administration to rats will eventually elicit a depressive effect on the cardiovascular system which seems to be mediated in part by a reduction in vascular tone secondary to inhibition of the sympathetic system. By contrast, our results do not

suggest that chronic nicotine treatment modifies vascular tone in the iliac tree, since relative differences in iliac vascular resistance between the NIC-C and NIC-T groups were unchanged with near maximal vasodilation (Fig. 3), a procedure which would attenuate between-group differences in resting vascular tone. Thus, the mechanism of the nicotine-induced reduction in iliac resistance observed in the present studies probably involved modification of the basic architecture of the iliac vascular network. Exactly how such changes might occur cannot be deduced from present data.

Summary. In summary these studies have demonstrated that chronic nicotine consumption by oral administration elicits a reduction in vascular resistance within the iliac circulation of the rat. The mechanism appears to involve a change in the vascular architecture as opposed to modification of vascular tone. By contrast long-term consumption of an equivalent amount of nicotine via tobacco smoke had no significant effect.

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