

# Prostaglandins and nicotine-provoked increase in cutaneous blood flow

The mechanism of topically applied methyl nicotine-induced local cutaneous erythema was studied in normal human subjects. Aqueous methyl nicotine (0, 0.1, 1.0, 10.0, and 100 mmol/L) was applied to the volar forearms in quadruplicate after oral pretreatments with 25 mg doxepin hydrochloride, 600 mg ibuprofen, 50 mg indomethacin, 975 mg aspirin, and lactose placebo. The cutaneous vascular response was monitored by laser Doppler velocimetry. Although doxepin did not affect the cutaneous vascular response to methyl nicotine, indomethacin, ibuprofen, and aspirin significantly suppressed the response. Because indomethacin, ibuprofen, and aspirin have different chemical structures, the common property of inhibition of the response to methyl nicotine may be assigned to their common pharmacologic action, i.e., inhibition of prostaglandin bioformation. (*CLIN PHARMACOL THER* 38:273-277, 1985.)

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Systemic administration of nicotinic acid leads to a generalized cutaneous erythema (flush) mediated at least in part by prostaglandin biosynthesis.<sup>29</sup> Topical applications of nicotinic acid and its esters provoke a local cutaneous erythema.<sup>20</sup> Because prostaglandins are involved in the cutaneous vasodilation provoked by systemic nicotinic acid, we undertook to examine further the mechanism of nicotine-provoked, local cutaneous vasodilation. Methyl nicotine was selected because it is readily soluble in water and penetrates rapidly<sup>20</sup> through the interstitial channels, bypassing the keratinized cells.<sup>1</sup>

## METHODS

Our subjects were 19 clinically normal human subjects (seven men, 12 women) aged 21 to 33 years. All subjects abstained from prescription and over-the-counter drugs both 10 days before and throughout the

study. Patches of coarse porosity, quantitative, ashless grade filter paper (1.23 cm<sup>2</sup>) saturated with 0.0, 0.1, 1.0, 10.0, and 100.0 mmol/L aqueous methyl nicotine were applied to the volar forearm. Each concentration was tested in quadruplicate with randomization of order and location of individual patches. Patches were sequentially applied for 5 minutes, removed for 5 minutes, and then the laser Doppler velocimeter probe was applied for 5 minutes. Preliminary studies\* disclosed that the maximal reaction occurred after 5 minutes. Challenges were performed with subjects in the sitting position after oral pretreatment with 25 mg doxepin HCl, 600 mg ibuprofen, 50 mg indomethacin, 975 mg aspirin, and lactose placebo. All subjects were given two capsules before each challenge; one capsule was taken 4 hours before and the other capsule 1 hour before each test. Doxepin or placebo were given 4 hours and ibuprofen, indomethacin, aspirin, or placebo were given 1 hour before topical methyl nicotine testing. Each of the pretreatment studies was performed on each subject at a minimum interval of 10 days. All oral pretreatments were given in a randomized, double-blind manner in opaque gelatin capsules. During the first half of the study, the unpleasant sedative effect associated with doxepin precluded adequate blinding. The data were then analyzed independent of the investigators directly conducting the study, and doxepin pretreatment was found to have negligible effects. Thus no more

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**Table I.** Adjusted cutaneous perfusion index (in millivolts)

Pretreatments	Methyl nicotine levels (mmol/L)				
	0.1	1.0	10.0	100.0	All levels
Placebo	20.0 ± 7.2	108.0 ± 17.6	232.8 ± 19.2	251.6 ± 18.4	152.0 ± 20.0
Doxepin	16.4 ± 10.0	135.2 ± 24.4	225.2 ± 26.4	226.0 ± 25.6	156.0 ± 26.4
Ibuprofen	6.8 ± 7.2	38.4 ± 17.6	87.6 ± 19.2	118.8 ± 18.4	64.0 ± 20.0
Indomethacin	6.4 ± 7.2	54.0 ± 17.6	64.4 ± 19.2	63.2 ± 18.4	48.0 ± 20.0
Aspirin	5.2 ± 7.2	12.8 ± 17.6	19.2 ± 19.2	17.2 ± 18.4	12.0 ± 20.0
Mean square error (analysis)	24.56	149.76	172.88	162.16	190.4

Data are  $\bar{X} \pm SE$ .

doxepin pretreatments were given during the rest of the study.

Vascular response to methyl nicotine after pharmacologic pretreatment was assessed by laser Doppler velocimetry<sup>3,8,19</sup> (Model LD 5000; MedPacific Inc.). The low-power (5 mW) helium-neon laser source emits light at 632.8 nm, which is delivered to the skin by flexible graded index fiberoptic light guides. A portion of the incident light strikes nonmoving structures and is reflected with no shift in frequency. The portion that strikes moving erythrocytes is reflected with a shift in frequency (Doppler broadening). The reflected light is conducted from the tissue surface through a second fiberoptic light guide, mixed (heterodyned), and analyzed in real time by an analog processor that provides a continuous output of the instantaneous mean Doppler frequency in the photocurrent identified by a square-law detector. The electrical analog of this output (in millivolts) is displayed digitally and on a chart recorder. While not a quantification of skin blood perfusion as volume/weight of tissue per unit time, laser Doppler velocimetry does produce data known to be related to fundamental skin perfusion characteristics, i.e., volume and flow rate,<sup>25</sup> referred to here as the cutaneous perfusion index.

Each of the five pretreatments in each of the 19 subjects were at five methyl nicotine levels: 0.0, 0.1, 1.0, 10.0, and 100.0 mmol/L. Because subjects differed significantly in baseline responses to water (0.0 mmol/L), each subject's response to water was subtracted from the response to nonzero levels of methyl nicotine. Except for comments on pretreatment effects on unstimulated cutaneous blood flow, all subsequent results are adjusted values, i.e., flow rate at a specified methyl nicotine level minus flow rate at 0.0 mmol/L methyl nicotine.

The methyl nicotine level was treated as a covari-

ate. To linearize the relationship of flow to methyl nicotine level, logarithms to the base 10 were taken of the methyl nicotine levels multiplied by 100. Analyses of each subject's results confirmed that this transformation gave a linear response, indicating that for most subjects the range of methyl nicotine levels was appropriately scaled.

Global analyses of the data were performed with the general linear model (GLM) of the Statistical Analysis System (SAS) package.<sup>15</sup> This enabled us to treat subjects as a random effect in the model and to test for different slopes of flow (adjusted) vs.  $\log(100 \times \text{methyl nicotine level})$  for different pretreatments, even though the design was unbalanced in that eight subjects did not receive doxepin. Because of problems of estimation in contrasts involving doxepin, the results that follow are taken either from a global analysis treating levels of methyl nicotine as a categorical variable or from separate analyses at each level.

## RESULTS

In a preliminary analysis of raw data at the control level (0.0 mmol/L), the mean flow for placebo was 83.6 mV. The three prostaglandin synthetase inhibitors gave mean values of 73.5, 73.5, and 60.6 mV for ibuprofen, indomethacin, and aspirin, respectively. Only results with aspirin differ significantly from those with placebo.

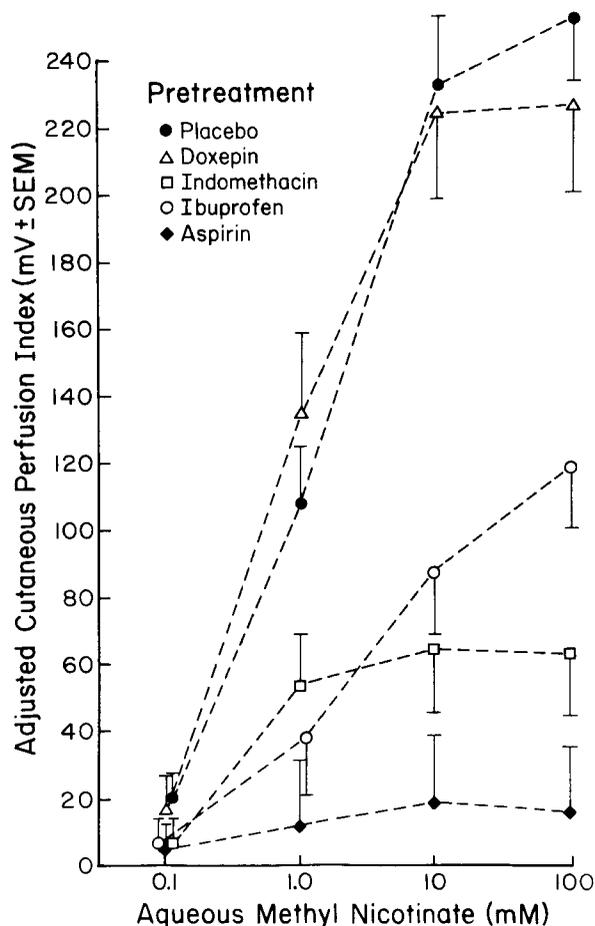
Results presented in Table I and Fig. 1 are mean adjusted flow. Over all levels the mean adjusted flow was not significantly different for placebo and doxepin or for ibuprofen and indomethacin. All other differences are significant at the 5% level, allowing for repeated tests of significance. Over the separate levels of methyl nicotine, there is a gradual trend from no significant differences at the 0.1 mmol/L level to two significantly different but statistically overlapping groups of pre-

treatments at the 1.0 mmol/L level: for placebo and doxepin and for ibuprofen, indomethacin, and aspirin. At the 10.0 mmol/L level these two groups do not overlap and are quite distinct. The results at the 100.0 mmol/L level are similar to the overall results for all levels combined. At 100.0 mmol/L, placebo and doxepin are not significantly different. However, a significant difference now exists among the three prostaglandin inhibitors, with aspirin having a significantly greater effect than either ibuprofen or indomethacin. Thus aspirin is clearly the most effective inhibitor, with ibuprofen and indomethacin being next effective in suppressing the vascular response. We found no significant differences between placebo and doxepin, results we do not think to be due to the reduced number of subjects taking doxepin. Although no attempt was made to quantify the erythema in response to topical methyl nicotinate, erythema was barely perceptible at low concentrations, especially after ibuprofen, indomethacin, and aspirin, and was most intense at high concentrations, especially after doxepin and placebo.

### DISCUSSION

Several aspects of our study deserve special comment. First, not only are the effects of methyl nicotinate on the cutaneous vasculature dose related as assessed by laser Doppler velocimetry, but also the doses of methyl nicotinate used were in the linear portion of the dose-response curve. This is compatible with the conclusions drawn from our unpublished pilot studies that these four doses represent the linear portion of the curve relating dose of methyl nicotinate to laser Doppler velocimetric response. Studies in the linear portion of the dose-response curve are important in detecting pharmacologic effects of antagonists. Second, the use of adjusted data is desirable because it imposes more conservative interpretation of the data. Since pretreatment with prostaglandin synthetase inhibitors was followed by less unstimulated cutaneous blood flow (significant only for aspirin), raw data will include this effect. It is more rigorous to look for a pretreatment effect after subtracting out the effect on baseline cutaneous blood flow.

We have shown that indomethacin, ibuprofen, and aspirin significantly suppressed the cutaneous vascular response to methyl nicotinate. Because these three drugs have different chemical structures, the common property of inhibition of the response to methyl nicotinate may be assigned to their common pharmacologic action, i.e., inhibition of prostaglandin bioformation. Since the systemic cutaneous vascular response to nico-



**Fig. 1.** Cutaneous vascular response to different concentrations of topically applied methyl nicotinate after oral pretreatment with placebo, indomethacin, ibuprofen, aspirin ( $n = 19$  for all), and doxepin ( $n = 11$ ).

tinic acid and the local cutaneous vascular responses to the methyl ester of nicotinic acid are suppressed by inhibitors of prostaglandin biosynthesis, the effect of the nicotinate moiety on vascular smooth muscle may be mediated by vasodilator prostaglandins.

In contrast, doxepin pretreatment did not affect the measured cutaneous vascular response despite a dosage schedule at which its antihistaminic activity in the cutaneous vasculature is demonstrable.<sup>21</sup> Also, doxepin is an antagonist at both  $H_1$ - and  $H_2$ -histamine receptors.<sup>16</sup> Thus histamine does not play a major role in methyl nicotinate-provoked local cutaneous erythema.

Because nicotinic acid and its esters (methyl, ethyl, butyl, hexyl, octyl, tetrahydrofurfuryl, and xanthinol) all provoke vasodilation,<sup>5,20,29</sup> it is reasonable to ascribe this pharmacologic activity to the common chemical

structure, the nicotinate moiety. Thus results of previous pharmacologic studies on the effects of nicotinic acid and its esters should be compatible with the present study and provide further support for our hypothesis. These data will be adduced according to category.

There are four categories of pharmacologic evidence supporting the role for a mediator, in this case, prostaglandins mediating nicotinate-provoked vasodilation. First, the mediator must be synthesized or stored in a manner such that it can act on the responding tissue. Not only are vasodilator prostaglandins, including prostacyclin, produced in blood vessels,<sup>13</sup> but in addition prostaglandin biosynthesis occurs in the skin.<sup>10,12</sup> Second, the mediator must be liberated (released from storage or immediately synthesized) in response to the pharmacologic agent. In fact, nicotinic acid stimulates prostaglandin synthesis in the isolated rabbit heart<sup>11</sup> and in rat platelets.<sup>26</sup> Third, there must be identity of action, i.e., either the mediator itself or a stable analog of the mediator must reproduce the effect of the pharmacologic agent. The topical application of 16-vinylprostaglandins leads to a similar erythema, restricted to the area of application and not associated with pain, swelling, or itching.<sup>2</sup> The systemic administration of a variety of prostaglandins leads to flushing indistinguishable from that provoked by nicotinic acid.<sup>27,28</sup> The fourth category is the documentation of specific antagonism.

We have previously shown that aspirin blocks nicotinic acid-induced flush.<sup>29</sup> Aspirin also inhibits the vascular response to topically applied tetrahydrofurfuryl nicotinate.<sup>30</sup> Furthermore, indomethacin inhibits nicotinic acid-induced flush in man.<sup>23</sup> However, indomethacin may be a vasoconstrictor as well as an inhibitor of prostaglandin biosynthesis.<sup>4</sup> Thus other prostaglandin synthetase inhibitors should also be studied to discriminate between these two activities. In our study all three chemically dissimilar prostaglandin biosynthesis inhibitors antagonized the nicotinate-provoked vascular response.

Moreover, doxepin, which has potent antihistaminic effects, did not affect the nicotinate vascular response. In previous studies neither the iontophoresis of tripelennamine HCl, an antihistamine, nor the injection of compound 48:80, a mast cell histamine depleting agent, led to inhibition of the erythema provoked by tetrahydrofurfuryl nicotinate.<sup>14,30</sup> Furthermore, should histamine and other mast cell products be involved, one would expect itching and a burning, painful sensation, which do not occur after nicotines.

The significance of the hypothesis that nicotinate-

provoked vasodilation is mediated by prostaglandins includes both basic and clinical aspects. It is important to note that the effect of nicotines on the blood vessels need not be direct. This could explain the "conduction mechanism" proposed after observing vasodilation in the hamster cheek pouch extending beyond the immediate vasculature in contact with droplets of nicotinate esters.<sup>6</sup> Similarly, although others infer that it is nicotinate that is rapidly transported radially in the dermis,<sup>7</sup> it could be prostaglandins. Certainly, the end point of erythema is insufficient to distinguish between these two possibilities. In fact, since flushing occurs only while the nicotinate concentration in blood is rising and disappears when a constant level of nicotinic acid is maintained,<sup>22</sup> the stimulus for prostaglandin bioformation may be the rate of increase, and not simply the absolute level, of nicotinate. This relationship would invalidate conclusions regarding dermopharmacokinetics,<sup>24</sup> which assume first-order kinetics and a direct relationship between vascular effect and nicotinate presence. Finally, the use of such topical and systemic agents to provoke prostaglandin biosynthesis and vasodilation will be an additional pharmacologic tool in the study of cutaneous and systemic vascular mechanisms and reactivity. Vasodilator prostaglandins may be important in the maintenance of baseline cutaneous blood flow, since we found that unstimulated flow was lower after pretreatment with prostaglandin synthetase inhibitors (significant only after aspirin pretreatment).

The clinical significance of this hypothesis will require further work. Indeed, several directions can be suggested. Topical nicotinate challenge leads to an "atypical" or blanch reaction in atopy,<sup>14,17</sup> psoriasis, rheumatoid arthritis, and dyshidrotic eczema<sup>17</sup> and "nonreactivity" in scleroderma and diabetes mellitus.<sup>14</sup> The diminished reactivity of patients with diabetes might be related to impairment of the vessel wall's ability to manufacture prostacyclin, since this defect has recently been shown in patients with diabetes and vasculopathy.<sup>9,18</sup> The nicotinate reaction must be reevaluated in these various disorders, with an active search for the role of prostaglandin biosynthesis.

In conclusion, previous studies have shown that prostaglandin bioformation occurs in skin and blood vessels; that nicotines provoke vasodilation; that nicotines provoke prostaglandin biosynthesis *in vitro*; and that prostaglandins and stable prostaglandin analogs provoke a qualitatively identical vascular response to that provoked by nicotines. We have shown that specific antagonism of prostaglandin biosynthesis inhibits nicotinate-provoked vasodilation in human skin. Thus the

nicotinate-provoked vasodilator response is mediated, at least in part, by prostaglandin biosynthesis.

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