Noninvasive Assessment of Local Nicotinate Pharmacodynamics by Photoplethysmography

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The local pharmacodynamics of a topical vasodilator (methyl nicotinate) has been followed noninvasively using photopulse plethysmography. This technique is sensitive to changes in blood flow through the cutaneous microcirculation and responds to the pharmacologic stimulus of the vasodilatory agent employed. Five different application sites for the drug were studied and the time course of the local effect (i.e., onset, duration, and decay) was recorded. The applied amount of drug elicited, within a short period, a response which was saturable such that the observed increase in blood flow reached a plateau level. The decay of the elevated perfusion required approximately 1 h, suggesting a half-life for elimination of the drug from the skin of about 10 min. This result agrees closely with other reported values and suggests that the pharmacodynamic measurements of this study may prove useful in elucidating aspects of dermal pharmacokinetics.

Information about the time course of drug behavior at and within the region of topical application is of considerable importance to the rational design of dermatologic therapy for use in the clinical situation. A convenient way to investigate in vivo the cutaneous action of vasodilatory or vasoconstrictive drugs is through their effects on the cutaneous microcirculation. Among the currently available techniques [1], which include direct visualization, plethysmography in its various forms, thermal measurements, dye techniques, radioisotope clearance methods, and laser Doppler spectroscopy [2], photoplethysmography (PPG) [3] offers certain distinct advantages over many of the alternative methods. In PPG, a small probe that
contains a light source and a photodetector is attached to the skin surface. Light from the source enters the skin and is then multiply scattered by the skin back into the detector. The wavelength of incident radiation used in PPG lies in the range of 800–900 nm where the transmission through the skin is large compared with blood. PPG is commercially available at prices much lower than laser Doppler velocimeters. Although the results cannot be reliably quantified in the absolute sense (it is even not clear whether more reflected light indicates more or less blood [4]), this same objection holds for most other methods as well [1,5]. PPG has a long history [3] and most recently has been applied successfully to screen patients with significant obstruction of the extracranial carotid artery [6,7,11], to assess and differentiate between superficial and deep insufficiency in the lower leg [8], and to evaluate the healing potential of skin ulcers [9]. In the pharmacologic context, both vasodilatory and vasoconstrictive topically applied agents have been studied using PPG [3]. Cummings [10] investigated the temperature and concentration effects on the initial stages of the penetration of N-ocytamine through the human skin, while Thune [11] measured the full time course of vasoconstrictive steroids in normal and stripped skin and found it to be on the order of several days. Here, we have employed PPG to monitor both the onset and decay of the effect of methyl nicotinate (3-pyridine carboxyl acid, methyl ester) absorbed through the skin. Nicotinic acid and its esters are potent topical vasodilators, rapidly eliciting a distinctive erythema after application [12]. Indeed, the onset of erythema often has been used as a convenient physiological and point with which to quantify the skin absorption of these compounds [13]. Subsequent to the appearance of redness, the erythematous area (i) spreads radially to a maximum (and increases in intensity) before (ii) gradually fading away. The former process has recently been visually measured and interpreted [14]. The latter, however, involves too much subjectivity to be so treated. It seems reasonable to expect that the rate of disappearance of erythema will be closely related to the removal of drug by the dermal capillaries into the systemic circulation. In terms of the nicotinate life-time within the skin, therefore, this event is particularly pertinent. Because PPG is able to reflect alterations in skin blood flow, as shown by the works of Hertzman and associates [15–20], it seemed likely that the technique would prove capable of providing an indication of both the arrival and removal of methyl nicotinate into and from the skin.

We report noninvasive experiments using PPG which allow us to assess the local pharmacologic effect of methyl nicotinate following topical application. Data are presented for the time course of drug penetration at various sites on the body from the time of application up to the return of the photoplethysmographic response to its baseline level.

**MATERIALS AND METHODS**

Methyl nicotinate (1 m aqueous solution) was topically applied to the upper and lower forearms, upper and lower back, and shins of 3 volunteers. Each experiment included simultaneous recording of 2 sites, usually bilateral and symmetrical. Every chosen site was first recorded with the photoplethysmograph (see below) to indicate the baseline and thus to eliminate sites above ectasia or veins, which give rise to high readings and lower sensitivities. Each site was exposed to the methyl nicotinate for 15 min through a saturated Inseco Ab (Sweden) (Al Test) 1 mm-diameter test patch and then cleansed with a tissue paper. The skin was not pretreated or cleansed. A Medasonics PPG13 photoplethysmograph was used with a PH77 reflectance sensor. This small rectangular sensor (2.1 x 1.0 x 0.7 cm) houses a light-emitting diode and a photodetector which detects a portion of the transmitted light that was reflected from blood in small superficial vessels. The electrical output of the phototransducer is, therefore, amplitude modulated according to the changing volume of blood in the microcirculation. To ensure repeatability, we used the PPG13 in its arterial mode in which slow changes in venous or regional blood flow are not detected and only the rapidly changing pulsatile signals produced by arterial blood volume changes in the microcirculation are amplified (with a 0.5–16 Hertz frequency response). The simultaneous outputs of 2 sensors were recorded on a R12A Medasonics chart recorder. After equalizing the sensors' outputs with the aid of a Medasonics calibrator, the sensors were placed on the chosen sites using a transparent double-sided tape.

The whole procedure for drug application and probe positioning at the 2 sites took less than 1 min. A continuous recording was taken for the first 10 min, followed by 30-s recordings every 5 min.

We also recorded the PPG response following intradermal injection of (i) 50 μl of 1 x 10⁻⁴ m methyl nicotinate solution in normal saline, (ii) 50 μl of 1 x 10⁻⁴ m methyl nicotinate solution in normal saline, and (iii) 50 μl of normal saline into the flexor aspect of the forearms of subject 1.

All experiments were performed in a single well-ventilated room at a temperature of 22 ± 2°C and at a relative humidity in the range of 50–70%.

**RESULTS**

Typical recorder outputs from the upper forearm appear in Fig 1. It is readily seen that while the amplitude of the volume pulsations of the microcirculation blood does not change appreciably immediately after the application of the methyl nicotinate (Fig 1a,b), a substantial amplitude increase follows (Fig 1c) with a subsequent decay (Fig 1d) to the baseline level.

Fig 2 depicts the time dependence of the peak–to-peak amplitude of the pulsations as determined from samples of the recorder output for the upper forearms of 1 of the subjects, (left forearm, right forearm). The figure shows the pulse wave amplitude, PWA, as well as the ratio PWA baseline

Similar measurements of the remaining 2 volunteers produced qualitatively the same results. The pattern of response seen at the other 4 sites tested (lower forearm, upper and lower back, and shins) was consistent with that shown in Fig 2.

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*The incident light of the source is reported to penetrate to a depth of 1–1.5 mm (Medasonics, technical information)."
Fig 2. Time dependence of the peak-to-peak amplitude, PWA, upper forearm. a, Direct reading from recorder output. b, The natural logarithm of the ratio PWA. Solid line: left upper forearm. Dotted line: right upper forearm.

Fig 3. Time dependence of the peak-to-peak amplitude, PWA, following injection of saline to the left lower forearm (solid line) and 50 μl of methyl nicotinate to the right lower forearm (dotted line, 1 × 10^{-3} M; dotted-dashed line, 1 × 10^{-4} M). a, Direct reading from recorder output. b, The natural logarithm of the ratio PWA.

The applied amount of nicotinate perturbs the local microvasculature in an approximately equal fashion. The differences that we find among "resting" (no drug) PPG responses, i.e., blood flow, at the various positions are in general agreement with older data [15,19]. Currently in our laboratory, further work is being undertaken to more clearly reveal the regional variation of blood flow in the dermal circulation.

Each of the response curves, having risen rapidly, reaches a plateau region. This observation resembles that reported by Cummings [10] for the penetration of N-octylamine, although it should be pointed out that the latter compound has a different mechanism of action from that of methyl nicotinate. The character of the response seen in our studies suggests that the quantity of vasodilator absorbed in a 15-s application is sufficient to produce a saturating effect. This is true at each position studied and is not influenced by the magnitude of the baseline PPG response detected.

It is a reasonable postulate that the observed effect caused by methyl nicotinate is related to the amount of the compound present at the site where it elicits its action. We may speculate that the relationship between effect and amount is a function of the number of blood vessels accessible to the drug, i.e., the number of active sites or receptors, and the degree to which each vessel has been, or has the potential to be, dilated.

It follows that, in a qualitative sense, we are able in the above fashion to provide a simple, acceptable explanation of the
The intradermal injections were undertaken in this study to provide a sensible "control" system and, subsequently, to acquire further into the apparent generality of the rate of the \( k \) process. Several features of the data in Fig 3 are of interest. Firstly, we see a cutaneous blood flow response to injection trauma because there is a significant change in PPG output following administration of saline. This effect has been reported previously by Holloway [5] using laser Doppler velocimetry. Secondly, the response decay after intradermal delivery of nicotine parallels closely that found following a topical application. It is clear, therefore, that methyl nicotinate is able to relatively rapidly cross human stratum corneum. Thirdly, the 3 intradermal injections of drug (50 \( \mu l \) of \( 1 \times 10^{-5} \) m and 50 \( \mu l \) of \( 1 \times 10^{-4} \) m) correspond to the introduction of 6.85 \( \mu g \) and 0.685 \( \mu g \) respectively. Obviously, there is not a 10-fold difference in the microcirculation response as recorded by PPG. It follows that, at present, it is very difficult to correlate the magnitude of the PPG response with a discrete amount of drug at the active site(s). The problem may well be related to the "saturation" phenomenon discussed earlier. To probe the relationship between response and amount of drug clearly requires further investigation in which applied drug concentration and skin contact time and area are important variables. The availability of other nicotinic acid esters of different physicochemical characteristics (e.g., size, solubility, lipid/water affinity), yet consistent pharmacologic effects [11], offers another route to study the potential connection among our pharmacodynamic measurements, drug availability at effectors, and eventually, it is hoped, the percutaneous absorption process itself.

In conclusion, it appears that the PPG technique can monitor the time course of vasoactive drug behavior in the local region of skin to which the compound is applied. A simple treatment of the results has allowed an aspect of cutaneous pharmacokinetics to be addressed and the half-life for drug elimination to be estimated. We emphasize that the latter calculation implies a direct relationship between drug effect and drug presence, absolute confirmation of which is an objective pursued in our ongoing research.

REFERENCES

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Characterization of α- and β-Adrenergic Agonist Stimulation of Adenylate Cyclase Activity in Human Epidermal Keratinocytes In Vitro*

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Adrenergic receptors are responsible for selective recognition and binding of catecholamines and may in turn have an effect on epidermal cell growth and maturation via adenosine-3',5'-monophosphate (cAMP). Using endogenous catecholamines and drugs specific for α- and β-receptor subtypes, we have characterized the adrenergic receptor coupled to adenylate cyclase in cultured human epidermal keratinocytes. The relative potency order of stimulation of adenylate cyclase was: isoproterenol > epinephrine > norepinephrine. The predominant adrenergic receptor is of the β1-subtype, as also confirmed by the selective antagonists propranolol, butoxamine, and atenolol. No evidence of α-receptor mediation of adenylate cyclase was noted with the α-specific agonist, clonidine. Phenylephrine, the α-specific agonist, affected cAMP formation but this response could not be totally inhibited with prazosin, suggesting an unknown mechanism of action. Human keratinocytes retained the same β-receptive receptor potency order properties throughout growth and maturation.

In many mammalian systems cyclic AMP affects cellular metabolism by processes leading to growth enhancement or restriction. Epidermal keratinocytes possess a series of cell surface receptors for catecholamines [1], histamine [2], adenosine [3,4], and prostaglandins E1 and E2 [5] which are coupled to adenylate cyclase. While species differences do occur in the magnitude of response and order of sensitivity to a variety of agonists, we previously have shown that the adenylate cyclase of human keratinocytes is most sensitive to agents that stimulate the catecholamine receptor [6]. That this receptor may be important with regard to regulation of epidermal growth is suggested by the fact that several laboratories have independently reported that involved psoriasis epidermis in vitro has a decreased response to catecholamines [7-9], which has been confirmed by cytochemical analysis [10]. Also, in tissue slices of epidermis that had been treated with hexadecane to produce hyperplasia, the increased proliferation, amino acid incorporation, and glycolysis were associated with loss of responsiveness to β-receptive agonists [11].

Adrenergic receptors are responsible for selective recognition and binding of catecholamines and may mediate quite distinct cell functions. Beta-adrenergic receptors have been identified on membrane preparations of numerous mammalian cells including rodent and human skin cells [12-14]. In this communication we focus on characterization of the receptor subtypes coupled to adenylate cyclase in human epidermal keratinocytes grown in vitro. Alpha- and β-adrenergic receptor subtype clas-