Clonidine induces nitric oxide- and prostaglandin-mediated vasodilation in healthy human skin

Daniel Hermann, Tanja Schlereth, Thomas Vogt, and Frank Birklein

Department of Neurology, University of Mainz, Mainz, Germany

Submitted 7 March 2005; accepted in final form 18 July 2005

Hermann, Daniel, Tanja Schlereth, Thomas Vogt, and Frank Birklein. Clonidine induces nitric oxide- and prostaglandin-mediated vasodilation in healthy human skin. J Appl Physiol 99: 2266–2270, 2005—First published July 21, 2005; doi:10.1152/japplphysiol.00271.2005.—Sustained sympathetic activation not only leads to vasoconstriction but also might induce paradox vasodilation. This study was performed to explore whether and how α₂-receptor stimulation mediates this vasodilation. We investigated 11 healthy subjects in 33 dermal microdialysis (MD) sessions. After nerve trunk blockade, MD fibers were inserted and perfused with physiological saline until skin trauma-related vasodilation subsided. Thereafter, fibers were perfused with either clonidine solutions (10⁻⁵, 5 × 10⁻⁵, 10⁻⁴ mol/l), N⁶-monomethyl-arginine (L-NMMA; nitric oxide synthase blocker), acetylsalicylic acid (ASA; cyclooxygenase blocker), or combinations of these. Laser-Doppler scanning of the investigated skin revealed that clonidine not only induces vasoconstriction but subsequently also vasodilation with higher concentrations (P < 0.001). In contrast, both L-NMMA and ASA induced vasoconstriction (P < 0.001). By coapplication of 10⁻⁴ mol/l clonidine with L-NMMA or ASA, vasodilation was partially prevented (P < 0.001). Our results demonstrate that sustained α₂-receptor stimulation induces vasodilation in a dose-dependent way, which is mediated by nitric oxide and prostaglandin mechanisms in human skin.

MATERIALS AND METHODS

Subjects. Eleven healthy volunteers (all men) with an average age of 25.5 yr (range 24–29 yr) were investigated. All subjects participated three times, resulting in a total of 88 microdialysis (MD) fibers for perfusion analysis. The study was performed in a temperature (22°C)- and humidity (50% relative humidity)-controlled laboratory after a period of acclimatization of at least 30 min. The subjects’ arms rested at heart level and were fixed in a vacuum cushion. Skin temperature at the forearm was measured before and after each experiment using an infrared thermometer and was found to be constant (32.2 ± 0.24 and 32.1 ± 0.24°C, respectively). Written, informed consent was obtained from all participants, and the study was approved by the local ethics committee.

Dermal microdialysis. Dermal microdialysis was performed according to our previous study (38). In brief, the nerve trunk of the superficial branch of the radial nerve was blocked with 2% lidocaine. Thereby dorsal root reflexes and efferent sympathetic activity were blocked, and the fibers could be inserted pain free. Sterile MD fibers were inserted intradermally into the anaeasthetized skin to a length of 1.5 cm. Three to six plasmapheresis fibers (depending on the area of analgesic skin) were inserted, and a distance of at least 1 cm was kept between each fiber. Some more fibers than necessary were initially inserted, because there is always a risk that some MD fibers might not operate properly. MD fibers were perfused with only one drug concentration. During the baseline period, which lasted 1 h, fibers...
were perfused with physiological saline employing a microdialysis pump (model PHD 2000, Harvard Apparatus) and a constant flow rate of 4 µl/min.

In a first series of experiments after the baseline, we switched the perfusion medium to CL (Sigma-Aldrich, Munich, Germany) dissolved in saline for a period of 30 min, at a concentration of 10⁻³ mol/l (total of n = 11 fibers), 5 × 10⁻⁴ mol/l (n = 11 fibers), or 10⁻⁴ mol/l (n = 11 fibers). Because local vasodilation occurred only with higher concentration of CL (see RESULTS), in a second series of experiments we applied N¹-monomethyl-L-arginine 10⁻² mol/l (L-NMMA; Sigma-Aldrich, Munich, Germany) or 10⁻² mol/l acetylsalicylic acid (ASA; Bayer, Leverkusen, Germany) in saline for 30 min. Thereafter, in a third series of experiments, we mixed either L-NMMA or ASA with 10⁻³ mol/l CL (n = 11 each).

Laser-Doppler imaging. Laser-Doppler imaging (LDI) technique (Moor Instruments, London, UK) was used to obtain pictures of skin perfusion. A rectangular area covering the skin where the MD fibers were inserted was scanned. Each scan area was individually adjusted. Scans took 59.8 ± 2.1 s on average, and scan speed was 4 ms/pixel. LDI pictures were recorded every 15 min during the baseline period and every 4 min during the stimulation period.

On the basis of visual screening of LDI pictures, we made the decision to quantify skin blood flow in a rectangular area (2 × 10 mm) directly above the MD fiber. The mean blood flow in perfusion units was calculated using dedicated software offline (Moor LDI image processing, Moor Instruments). For statistical analysis, flux values were normalized and expressed as percent change of baseline (last picture before stimulation).

Data analysis. For statistical evaluation, multivariate ANOVA for repeated measures (7 pictures during drug application) was calculated. Main factors were the course of vasoconstriction (subsequent pictures, within-subject factor), the different concentrations of CL (10⁻³, 5 × 10⁻⁴, 10⁻⁴ mol/l), and the effects of L-NMMA and ASA (between-subjects factors). To allocate significant differences, Scheffé’s post hoc test was performed. For planned comparisons of single time points, one-way ANOVAs with Scheffé’s post hoc tests were calculated. In the paper, all values are presented as means ± SE. Statistical significance is considered at P < 0.05.

RESULTS

CL-induced vascular effects. Insertion of the MD fibers caused skin trauma, and related vasodilation was observed. During baseline perfusion with saline, this vasodilation steadily subsided and skin blood flow reached a plateau after 60 min, as has been described previously (38). Then, perfusion medium was switched to different CL solutions. CL at concentrations of 10⁻³, 5 × 10⁻⁴, and 10⁻⁴ mol/l initially (4-min picture) induced vasoconstriction, which did not differ between the different concentrations (n = 11; 10⁻³ mol/l: 39.5 ± 3.1%, 5 × 10⁻⁴ mol/l: 44.2 ± 3.6% and 10⁻⁴ mol/l: 34.3 ± 3.1%; F = 2.33, not significant).

Thereafter, blood flow curves diverge (F = 18.23, P < 0.001). CL at the higher concentrations (10⁻³, 5 × 10⁻⁴ mol/l) induced vasodilation (after 28 min: 10⁻³ mol/l, 76.2 ± 6.4%: 5 × 10⁻⁴ mol/l, 55.2 ± 5.7%; 10⁻⁴ mol/l, 29.7 ± 2.4%). Post hoc analysis of the whole curve revealed significant differences between 10⁻⁴ mol/l CL and both 10⁻³ mol/l CL (P < 0.001) and 5 × 10⁻⁴ mol/l CL (P < 0.005). Planned comparisons revealed that in addition there were significant differences between 10⁻³ and 5 × 10⁻⁴ mol/l CL from 20 min on (20 min, P < 0.03; 24 min, P < 0.05; 28 min, P < 0.03). For details, see Figs. 1 and 2.

L-NMMA- and ASA-induced vascular effects. Both L-NMMA (51.6 ± 4.3% after 28 min) and ASA (53.9 ± 5.0% after 28

![Fig. 1. Photograph of microdialysis fibers inserted in the skin (left) and the corresponding laser-Doppler picture (right) showing an increase of skin blood flow directly at the 10⁻³ mmol clonidine (CL) application site (bright pixels). Rectangle indicates area of skin blood flow quantification.]
However, no significant difference between CL/H11001 blood flow compared with saline (Fig. 3). Post hoc tests revealed significant differences between saline and l-NMMA (P < 0.001) and saline and ASA (P < 0.005).

**Inhibition of CL-induced vasodilation by l-NMMA and ASA.** For the investigation whether l-NMMA or ASA affects CL-induced vasodilation, we focused on 10^{-3} mol/l CL perfusion, because this concentration revealed greatest effects. Simultaneous perfusion of CL with ASA or l-NMMA partially prevented vasodilation (F = 9.31, P < 0.001; Fig. 4). After 28 min, the values were 76.2 ± 6.4% for CL, 53.3 ± 5.6% for CL + l-NMMA, and 67.0 ± 7.0% for CL + ASA. Post hoc test revealed significant differences between CL and CL + l-NMMA (P < 0.001) or CL + ASA (P < 0.05). There was, however, no significant difference between CL + l-NMMA and CL + ASA.

**DISCUSSION**

The present investigation shows that the α2-agonist clonidine (CL) not only induced vasoconstriction but also vasodilation in healthy human skin, in vivo. Vasodilation was dose dependent and restricted to skin areas with highest CL concentrations close to the MD membranes. Blocking NO synthase (NOS) by NMMA and cyclooxygenase (COX) by ASA suggested that both NO and prostaglandins contribute to CL-induced vasodilation in human skin.

**CL-induced vasoconstriction.** Our results indicate that CL delivered to the interstitial space by dermal MD first induces vasoconstriction. This vasoconstriction is of the same intensity as when phenylephrine (an α1-receptor agonist) was delivered (38). That means that CL from MD fibers first binds to α2-receptors on smooth muscles of arterioles, which mediate vasoconstriction and skin blanching. This is in accordance with former in vitro studies on human subcutaneous resistance vessels showing that postsynaptic vasoconstrictive α2-receptors are present on arterioles (29). Although newer studies in general revealed a predominance of adrenergic α1-receptors compared with α2-receptors mediating vasoconstriction in humans, obviously the α2-receptor component crucially depends on the investigated vascular bed (16). In the skin, α2-mediated vasoconstriction is very important (7, 35), because temperature is able to modify the α2-receptor response. This is significant for thermoregulation. Therefore, we performed our experiments on acclimatized subjects and under constant temperature conditions.

**CL-induced vasodilation.** After initial vasoconstriction, continuous delivery of CL then leads to vasodilation already after a few minutes in the present study. The switch from vasoconstriction to vasodilation strongly depends on CL concentration in the perfusate. The concentration of 10^{-3} mol/l CL did not induce vasodilation, 5 × 10^{-4} mol/l CL induced moderate, and 10^{-3} mol/l CL induced more pronounced vasodilation. This means the concentration range between very different reactions, maximum vasoconstriction and maximum vasodilation, is narrow. Clinically, CL is used as an antihypertensive drug. It is shown that CL preferentially activates central postsynaptic and peripheral presynaptic α2-receptors (22) and thereby reduces sympathetic activity (21). More recently, it has been demonstrated that by binding to endothelial α2-D receptors CL also induces vasodilation, and that this might amplify the antihypertensive action of CL (13). The vasodilatory effect of activation of endothelial α2-receptors has been demonstrated in rats (3) and pigs (2). The most important second messengers mediating vasodilation in humans might be NO (5) and prostaglandins (30) (28). The endothelial α2-receptor is a G protein-coupled receptor, which activates endothelial NOS, and NO is produced from L-arginine (12, 25). When NO is released from endothelium cells, it diffuses to the vascular smooth muscles and mediates vasodilation via cGMP, as has been shown for human skin (8). Consequently, intracellular Ca^{2+} and the sensitivity of the contractile system to Ca^{2+} decrease, leading to relaxation of the smooth muscle cells (6). The exact mechanism of α2-receptor coupling to prostaglandin synthesis is not clear. However, it is known that prostaglandins are produced in endothelial cells by activation of phospholipase A2, COX, and prostacyclin synthase. After diffusion, prostaglandins mediate relaxation of smooth muscle cells and thereby induce vasodilation, which again is Ca^{2+} dependent (37).
According to our results, both mechanisms significantly contribute to vascular tone at rest. Otherwise, the significant reduction of skin blood flow during inhibition of endothelial NOS by L-NMMA and during inhibition of COX by ASA could not be explained. Moreover, NO and prostaglandins might also contribute to CL-induced and α2-receptor-mediated vasodilation. In our study, inhibition of NOS reduced CL-induced vasodilation by 22.9% and inhibition of COX by 9.2%. Although we found no statistically significant difference, it is likely that NO is more important. Unfortunately we did not simultaneously apply L-NMMA and ASA, because adding up both inhibitory effects (see Fig. 4) might theoretically explain CL-induced vasodilation. Therefore, we must discuss further mechanisms that could contribute to CL-induced vasodilation.

First, CL binds not exclusively to α2-receptors but also to α1-receptors. Although α1-receptor-mediated vasodilation has not been shown in humans, more specific α2-receptor agonists like medotomidine or UK-14304 (17, 26) would have been advantageous. Second, high doses of CL, similar as it has been shown for high doses of phenylephrine (34), might exert some β-receptor-mediated vasodilation. However, unlike phenylephrine, CL is an imidazoline and usually has no pharmacologically relevant β-adrenergic affinity. Third, CL binds to peripheral presynaptic α2-receptors and thereby might decrease the release of norepinephrine from peripheral sympathetic neurons (34). Because our investigations were performed under nerve trunk blockade, which outlasts the MD experiments, this explanation is also very unlikely to explain our results. Fourth, CL interacts not only with α-receptors but also with imidazoline receptors (9, 20, 31). Located in the rostral ventrolateral medulla, imidazoline-receptor binding contributes to the antihypertensive effect of CL (10). Interestingly, imidazoline-receptor subtypes were also found in peripheral blood vessels (32). However, the role of imidazoline receptors in regulation of local blood flow in human skin remains unclear (33). Fifth, a very different mechanism of vasodilation in the skin depends on the activation of primary afferent nociceptive fibers, which then release vasodilator neuropeptides: the flare response (24).

In previous studies, it has been shown that C-fiber excitation might occur during catecholamine iontophoresis (11). Accordingly, one might speculate that CL binds to and stimulates α2-receptors on primary afferent neurons and thereby induces flare vasodilation. There are, however, some arguments against this hypothesis. The morphology of a flare would be quite different from the vasodilation observed in our study. Vasodilation in our study appeared as a reddish line (bright line in Fig. 1) corresponding to the highest CL concentration in the close vicinity of microdialysis membranes. In contrast, a flare would spread into the whole innervation territory of related axons (18). Furthermore, intradermal application of α-agonists into healthy human skin is not painful (38). After nerve injury, however, this might be basically different because in such patients sympathetically maintained pain has been demonstrated (19). Finally, we must consider that the attenuation of the CL response by L-NMMA or ASA could be simply the coincidence of vasodilation and vasoconstriction, which does not depend on a causal relationship. This, however, would be in contrast to previous animal and human data (13).

In a companion study, we investigated the role of adrenergic receptors in the flare response, and consistent with the present investigation we also did not detect adrenergic-receptor-induced flare responses (38). In this previous investigation using comparable methods, we applied different doses of epinephrine, phenylephrine, and CL. In that study, we also measured adrenoceptor-induced vasoconstriction but did not detect vasodilation after the application of 10−3 mol/l CL. At the first glance, this might be a contradiction. There are, however, good arguments to rebut this contradiction. The vascular reaction induced by CL obviously depends on the amount of CL that is delivered to the tissue. Both studies were performed under similar conditions, but we used differentially prepared (e.g., sterilized) MD membranes. This suffices to slightly affect CL tissue concentrations. As explained above, concentration range inducing vasoconstriction or vasodilation is narrow. Therefore, both studies are not really comparable, which is not a drawback because we never intended to compare both investigations.

Independent of the exact mechanisms, it is remarkable that CL is able to induce vasodilation in human skin at all. Our data suggest a possible interaction of sympathetic vasoconstrictor mechanisms and endothelium-related vasodilation. In rats, coupling of sympathetic excitation and NO release from endothelial cells has been described for mesenteric (4) and skeletal muscle arterioles but not for skin vessels (14). Obviously, skin perfusion in hairy rodents is differently organized from skin perfusion in more or less hairless humans. One main question remains: whether or not sympathetic activation also leads to (probably less pronounced) endothelial vasodilation, as high doses of CL did in our study. We cannot answer this question with our data. However, if there would be really dual responses, it offers an explanation for vasodilation, which is sometimes observed in neuropathic pain despite an obvious increase in sympathetic outflow.

ACKNOWLEDGMENTS

We thank Stuart Turner for help with the manuscript preparation. This study contains parts of the MD thesis of D. Hermann, which will be submitted to the Faculty of Medicine, Johannes Gutenberg-University, Mainz, Germany.

GRANTS

This work was supported by Deutsche Forschungsgemeinschaft Grants Bi 579-1 and Bi 579-4.

REFERENCES

7. Chotani MA, Flavahan S, Mitra S, Daunt D, and Flavahan NA. Silent alpha(2C)-adrenergic receptors enable cold-induced vasoconstriction in