No α-adrenergic agonist-induced C-fiber activation in healthy human skin

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Zahn, Sebastian, Stefan Leis, Christoph Schick, Martin Schmelz, and Frank Birklein. No α-adrenergic agonist-induced C-fiber activation in healthy human skin. J Appl Physiol 96: 1380–1384, 2004. First published December 12, 2003; 10.1152/japplphysiol.00990.2003.—In healthy volunteers, flare responses induced by norepinephrine (NE) iontophoresis have been observed. However, as NE iontophoresis is a combined electrical and chemical stimulus axon, reflexes cannot be directly linked to pharmocological activity of NE. Different concentrations of NE, clonidine (CL), and phenylephrine (PE) (NE: 10⁻¹⁰–10⁻³ M; CL, and PE: 10⁻⁸–10⁻³ M) were applied via intradermal microdialysis fibers into the skin of healthy volunteers. Simultaneously, skin blood flow was visualized by laser-Doppler imaging scans and quantified in a vasoconstriction skin area directly above the membranes to control drug effects and in expected axon reflex vasodilation areas that were 0.75 cm apart. NE, PE, and CL caused dose-dependent vasoconstriction. However, neither in the presumed axon reflex areas (quantitative analysis) nor on laser-Doppler imaging pictures (qualitative analysis) were any vasodilation observed. Even at concentrations causing maximum vasoconstriction (10⁻³ M for any drug), no vasodilation was induced. Our results indicate that, in healthy human skin, exogenously supplied α-adrenergic agonists alone do not activate nociceptors sufficiently to induce axon reflex flare.

sympathetic nervous system; axon reflex; laser-Doppler imaging

IN HUMAN SKIN, VASOCONSTRICTION (VC) IS MAINLY CONTROLLED BY THE SYMPATHETIC NERVOUS SYSTEM AND THE RELEASE OF NEUROTRANSMITTERS LIKE NOREPINEPHRINE (NE) (15a). ON THE OTHER HAND, NOXIOUS ACTIVATION OF C FIBERS INDUCES VASODILATION, THE AXON REFLEx FLARE (19). THIS FLARE IS MEDITATED BY THE RELEASE OF VASOACTIVE NEUROPEPTIDES [MAINLY CGRP AND SUBSTANCE P (SP)] FROM NOCICEPTIVE NERVE TERMINALS (7, 16). DIFFERENT PAINFUL NOXIOUS STIMULI (CHEMICAL, HEAT, MECHANICAL, OR ELECTRICAL) ARE ABLE TO EXCITE DIFFERENT SUBGROUPS OF C FIBERS, WHOSE ACTIVATION DETERMINES SIZE AND INTENSITY OF THE FLARE RESPONSE (5). FURTHERMORE, IT HAS BEEN SHOWN THAT SUBTHRESHOLD-FOR-PAIN ACTIVATION OF C FIBERS IS ALREADY SUFFICIENT TO INDUCE AXON REFLEXES (21).

SYMPATHETIC ACTIVATION, AND EVEN INJECTION OF NE, USUALLY IS PAIN FREE. THEREFORE, IT WAS ASTONISHING THAT AXON REFLEX VASODILATATION WAS DETERMINED DURING IONTOPHORESIS WHEN NE WAS THE ION USED TO DELIVER CURRENT INSTEAD OF SALINE (11). ACCORDING TO THESE RESULTS, ONE MIGHT SPECULATE THAT NE BENDS TO AND STIMULATES RECEPTORS ON PRIMARY AFFERENT NERVES AND CONTRIBUTES TO C-FIBER EXCITATION AND AXON REFLEx VASODILATION IN HUMAN SKIN. HOWEVER, IONTOPHORESIS OF CATECHOLAMINES HAS DISADVANTAGES BECAUSE THE CURRENT ITSELF COULD ACTIVATE C FIBERS (10). TO OVERCOME THIS SHORTCOMING, WE ADMINISTERED NE AND OTHER α-ADRENERGIC AGONISTS VIA MICRODIALYSIS IN THE PRESENT STUDY. THEN, IF AXON REFLEXES WERE OBSERVED, IT WOULD SUPPORT THE HYPOTHESIS THAT CATECHOLAMINES MAY CONTRIBUTE TO THE AXON REFLEX RESPONSE PREVIOUSLY OBSERVED DURING IONTOPHORESIS. IN COMBINATION WITH LASER-DOPPLER IMAGING (LDI), MICRODIALYSIS HAS BEEN PREVIOUSLY SHOWN TO BE SENSITIVE TO ASSESS LOCAL SKIN BLOOD FLOW CHANGES (25, 27).

METHODS

SUBJECTS. IN THE MICRODIALYSIS STUDY, WE INVESTIGATED 30 HEALTHY VOLUNTEERS (10 WOMEN, 20 MEN), 31.4 (19–65) YR OF AGE. PARTICIPANTS DID NOT TAKE ANY DRUGS AFFECTING SYMPATHETIC NERVE ACTIVITY OR C-FIBER EXCITABILITY, SUCH AS β-BLOCKERS OR ANTI-EPILEPTICS. SUBJECTS WERE SEATED ON A RECLINING CHAIR AND REPOSED THEIR ARMS AT HEART LEVEL. BEFORE STARTING THE EXPERIMENTS, SUBJECTS WERE ALLOWED TO ACCLIMATIZE IN OUR TEMPERATURE (24°C) AND HUMIDITY-CONTROLLED (50% RELATIVE HUMIDITY) LABORATORY FOR AT LEAST 1 H. SKIN TEMPERATURE AT THE INVESTIGATION SITE WAS BETWEEN 32 AND 35°C IN EACH SUBJECT. BEFORE INVESTIGATION, ALL SUBJECTS GAVE THEIR INFORMED, WRITTEN CONSENT, AND THE STUDY WAS APPROVED BY THE LOCAL ETHICS COMMITTEE.

MICRODIALYSIS. BEFORE INSERTION OF THE MICRODIALYSIS FIBERS, NERVE TRUNKS OF SUPERFICIAL BRANCHES OF THE RADIAL NERVE PROXIMAL TO THE WRIST WERE BLOCKED WITH 2% LIDOCAINE. WE WATCHED CLINICALLY THAT THIS BLOCK PERSISTED THROUGHOUT THE EXPERIMENT. THEREBY, EFFERENT SYMPATHETIC NERVE FIBERS OR DORSAL ROOT REFLEXES WERE BLOCKED, AND THE MICRODIALYSIS FIBER COULD BE INSERTED PAIN FREE. THREE (OR FOUR, SEE BELOW) SINGLE PLASMAPHORESIS HOLLOW FIBERS (0.4 MM IN DIAMETER, CUT-OFF 3,000 KD; DERMAL DIALYSIS, ERLANGEN, GERMANY) WERE THEN INSERTED INTRACUTANEOUSLY INTO THE ANESTHETIZED SKIN ON THE BACK OF THE HANDS. INSERTION DEPTH WAS MEASURED BY ULTRASOUND IN PREVIOUS STUDIES AND FOUND TO BE 0.65 MM, ON AVERAGE (25). THE FIBERS WERE INSERTED AT A LENGTH OF 1.5 CM VIA A 25-GAUGE CAMILLA. DISTANCE OF AT LEAST 1.5 CM WAS KEPT BETWEEN EACH FIBER. THE HOLLOW FIBERS WERE PERFUSED WITH PHYSIOLOGICAL SALINE VIA A MICRODIALYSIS PUMP (PUMP 22, HARVARD APPARATUS, HOLLISTON, MA). THE FLOW RATE WAS 4.0 μL/MIN. THE DIALYSATE WERE COLLECTED IN CALIBRATED GLASS CAPILLARIES TO CONTROL PERFUSION AND TO MINIMIZE OUTFLOW RESISTANCE.

AFTER A BASELINE PERIOD OF 60 MIN OF SALINE PERFUSION, PERFUSION MEDIUM WAS SWITCHED TO NE, CLONIDINE (CL), OR PHENYLEPHRINE (PE) IN SALINE FOR A FURTHER 30 MIN. NE CONCENTRATIONS RANGED FROM 10⁻¹⁰ TO 10⁻³ M; CL AND PE (ALL SUBSTANCES FROM SIGMA, ST. LOUIS, MO) WERE APPLIED AT CONCENTRATIONS OF 10⁻⁸–10⁻³ M. ALL SOLUTIONS WERE FREELY PREPARED FROM STOCK SOLUTIONS (10⁻³ M, STORED AT −50°C, RENEWED EVERY WEEK) BEFORE EACH EXPERIMENT.

IN ALL SUBJECTS, THREE FIBERS WERE INSERTED IN PARALLEL (N = 90 FIBERS IN TOTAL) AND PERFUSED WITH DIFFERENT CONCENTRATIONS OF EITHER NE, CL, OR PE. IN NONE OF OUR SUBJECTS, AN ADDITIONAL FOURTH FIBER WAS INSERTED AND PERFUSED WITH SALINE FOR CONTROL. ALL NUMBERS IN RESULTS REFER TO THE NUMBER OF FIBERS.

LDI. SUPERFICIAL BLOOD FLOW WAS QUANTIFIED BY USING A LASER-DOPPLER SCANNER (LDI, MOOR INSTRUMENTS, DEVON, UK). LDI IMAGE SERIES WERE RECORDED AT 15-MIN INTERVALS DURING BASELINE PERFUSION (60 MIN) AND AT 4-MIN INTERVALS DURING SIMULATION PERIODS. THE SIZE OF LDI SCANNED...
RESULTS

Adrenoreceptor-induced VC. After insertion of the microdialysis membranes, trauma-related vasodilation gradually declined during the first 60 min until stable baseline was reached. This is demonstrated by the lack of a further blood flow reduction in the VC area with saline perfusion compared with a distant control skin area (data not shown). Perfusion with NE (total of n = 52 experiments) induced a decrease in superficial skin blood flow above the respective microdialysis fiber (concentration effect F = 3.005, P < 0.001). Post hoc analysis revealed significant differences between saline and 10^{-5} M CL (n = 3; 48.7 ± 7.7%) and 10^{-3} M CL (n = 8; 67.7 ± 4.7%). The other values [10^{-8} (n = 2, 87.1 ± 9.4%), 10^{-7} (n = 3, 91.7 ± 7.7%), 10^{-6} (n = 2, 68.3 ± 9.4%), and 10^{-4} M (n = 2, 61.1 ± 9.5%)] were not significant.

In a similar way, PE (total n = 18) induced VC (concentration effect: F = 2.9, P < 0.001). Post hoc analysis revealed significant differences between saline and 10^{-3} M PE (n = 6; 61.0 ± 6.3%). PE concentrations of 10^{-6} (n = 2, 80.0 ± 11.0%), 10^{-7} (n = 3, 101.5 ± 9.0%), 10^{-6} (n = 2, 65.4 ± 11.0%), 10^{-5} (n = 2, 58.5 ± 8.9%), and 10^{-4} M (n = 3, 60.2 ± 11%) failed to reach significant difference in the post hoc analysis.

There was no significant difference in VC induced by NE, CL, or PE (F = 0.421, not significant; multivariate ANOVA adjusted for different concentrations). Results are summarized in Fig. 2, A–C, respectively.

Adrenoreceptor-mediated axon reflexes. At a concentration of 10^{-3} M, NE (n = 9), CL (n = 8), and PE (n = 6) induced significant VC, which was not different among the three drugs (Fig. 3A). This clearly supraphysiological concentration was chosen to assess possible axon reflexes quantitatively. In the presumed axon reflex area, there was no significant difference in skin blood flow among saline (89.8 ± 3.7%), NE (81.6 ± 3.5%), CL (90.4 ± 3.9%), or PE (90.3 ± 4.5%) (F = 1.408, not significant) (Fig. 3B).

Moreover, we watched every single picture during our experiments. There were, in total, 210 LDI scans with drug administration. In none of these LDI scans could any vasodilation, suspicious for axon reflex vasodilation, be detected, neither in concentrations below nor in concentrations exceeding VC threshold.

DISCUSSION

The results of our study show that, even when applied at supramaximum concentrations for VC, NE and other α-adre-
negic drugs did not activate nociceptive C fibers sufficiently to produce axon reflex flares in healthy human skin. Thus we could not provide evidence for interaction between sympathetic efferent fibers and afferent nociceptors in healthy human skin.

In the present study, we used different α-adrenoergic agents: NE, CL, and PE. When applied via dermal microdialysis, these drugs dose-dependently induced VC, and, with concentrations of $10^{-3}$ M, maximum VC and skin blanching could be

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

Fig. 2. Skin blood flow in the VC areas during 30 min of perfusion with norepinephrine (NE; A), clonidine (CL; B), and phenylephrine (PE; C). Different concentrations are shown at the top. Flux values are expressed as percent change of baseline blood flow before stimulation. Control values (perfusion with NaCl) are also shown. Values are means ± SE. There was a significant ($P < 0.001$ each) dose-dependent vasoconstriction induced by NE, CL, and PE compared with saline. The slight decrease of skin blood flow with saline stimulation is unspecific and could be also observed in remote skin areas.

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achieved. VC was not different, despite the different affinity to α₃- and α₂-receptors of each drug. That means the microdialysis system in our study previously was able to deliver drugs quantitatively to blood vessels.

In neuropathic pains, NE may intensify pain and allodynia, in particular if these symptoms were alleviated before by sympathetic blocks (1, 26). In these cases, a pathological cross talk between sympathetic efferent and primary afferent fibers has been shown, either in dorsal root ganglia (18) or in the periphery (24). If sympathetic activation of afferent fibers is a major mechanism of neuropathic pain, it is labeled “sympathetically maintained pain” (3). Injection of NE into intact human skin, however, is not painful. Activation of the sympathetic nervous system usually helps to suppress pain perception. This difference between neuropathic and physiological reaction would be explained if α₁- and α₂-receptors on primary afferents were either newly formed, increased in number, or sensitized in response to axonal damage. To further study these different mechanisms of sympathetic-nociceptive interaction, efforts were undertaken to find human models of sympathetically maintained pain. The results were ambiguous. Physiological activation of the sympathetic nervous system had no impact on capsaicin-induced pain, neither in the skin (4) nor in muscles (13). On the other hand, thermal hyperalgesia, which frequently occurs after capsaicin injection, was enhanced if NE was coapplied by iontophoresis (8). Recently, even sensitization of heat-sensitive C fibers after injection of catecholamines has been demonstrated in normal skin without capsaicin pretreatment (14). Correspondingly, there was one study showing a current-dependent vasodilation after iontophoresis of NE in normal human skin (11). Inside a ring-shaped iontophoresis electrode, a current-dependent vasodilatation was observed, which was significantly greater than with saline. It must be attributed to an axon reflex mechanism, because it was restricted to the iontophoresis site, and vasodilation could be prevented by pretreatment with local anesthetics. If indeed there were constitutively expressed adrenergic receptors on primary afferent neurons, a rapid adrenergic responsiveness of C fibers after nerve lesion (2) and “sympathetically maintained” neuropathic pain would be better understood. However, the findings in the present study were converse. Even at supramaximum concentrations for local VC, neither NE, CL, nor PE induced axon reflex vasodilatation, neither in the vicinity of the microdialysis fibers nor somewhere on the LDI pictures. The most important difference between the previously reported findings and our results is the route of application of adrenergic agents. For iontophoreses, it has been shown that the current itself induces C-fiber activation and axon reflexes (15). Saline iontophoresis, for example, could increase permeability of superficial skin layers (10), and vascular and even sensory nerve function might become compromised. Furthermore, the iontophoretic current could disrupt the perineurium of nociceptors in the skin (12). Activation of C fibers is even more obvious when NE is applied by injection (14). These drawbacks of iontophoretic or injection techniques do not apply for dermal microdialysis. Although insertion of the fibers induces skin trauma as well, these inflammatory skin changes usually subsided after 1 h (22). Thereafter, all substances could be delivered atraumatically. As demonstrated by dose-dependent VC in this study and by previous results (6), there is a linear correlation between concentration of substances in the perfusion medium and delivery through the microdialysis fibers into the tissue. It could be argued that afferent fibers are located more superficially in the epidermis and, therefore, might be reached easier by iontophoresis compared with microdialysis. However, it is obvious that the nociceptors, which are responsible for the axon reflex, must have a close relation to the subepidermal vessels, because epidermal diffusion of CGRP is very limited (27). This microdialysis mode of dermal application was used to provoke axon reflex vasodilation by various inflammatory mediators, such as bradykinin, histamine, and prostaglandin E₂ (20, 22). As the physiological source of catecholamines in the skin is the endings of vasoconstrictor neurons close to skin vessels, application via microdialysis might resemble the physiological conditions even better than the epidermal application via iontophoresis. The fact that skin blood flow was even slightly decreased in the presumed axon reflex area indicates that we indeed quantified skin blood flow at the border of VC. However, our results do not entirely rule out the possibility that the adrenergic system may still be involved in excitation of afferent C fibers. The adrenergic system may lower thresholds for axon reflexes to another stimuli (e.g., iontophoretic current). This could explain algiesic and sensitizing effects when NE was iontophoresed (11), injected (14), or coapplied with capsaicin (8, 17). Furthermore, adrenergic-receptor-induced VC could augment axon reflex vasodilation, in particular if VC surrounds the flare area. In both studies claiming NE-induced axon reflex flares, a ring-shaped iontophoresis electrode was used (11, 28). In this cases, vaso- dilatory neuropeptidines from current-excited (iontophoreses) primary afferents may accumulate because they could not be sufficiently cleared from the skin (9).

In conclusion, our results demonstrate that there is no good evidence for direct interaction of sympathetic and nociceptive skin fibers under physiological conditions in human skin. Therefore, human models of sympathetically maintained pain have to be interpreted with care. The mechanisms of how sympathoafferent coupling leads to sympathetically maintained pain has to be explored in studies basically involving patients rather than normal subjects.

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