Acetazolamide-induced cerebral and ocular vasodilation in humans is independent of nitric oxide

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Kiss, Barbara, Susanne Dallinger, Oliver Findl, Georg Rainer, Hans-Georg Eichler, and Leopold Schmetterer. Acetazolamide-induced cerebral and ocular vasodilation in humans is independent of nitric oxide. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1661–R1667, 1999.—Acetazolamide, a carbonic anhydrase inhibitor, is used orally in the treatment of primary and secondary open-angle glaucoma and induces ocular and cerebral vasodilation. Several in vitro studies have shown that carbonic anhydrase pharmacology and the L-arginine-nitric oxide (NO) pathway are closely related. We investigated the role of NO in acetazolamide-induced vasodilation on cerebral and ocular vessels in 12 healthy subjects in the presence or absence of Nω-monomethyl-L-arginine (L-NMMA), a NO synthase inhibitor, and in the presence or absence of L-arginine, the precursor of NO. Acetazolamide was administered after pretreatment with either L-NMMA or placebo and either L-arginine or placebo. Pulsatile choroidal blood flow was assessed with laser interferometric measurement of fundus pulsation. In addition, mean blood flow velocity (MFV) in the middle cerebral artery (MCA) and ophthalmic artery (OA) was measured with Doppler sonography. Acetazolamide increased ocular fundus pulsation amplitude (FPA; +27%, P < 0.001) and MFV in the MCA (+38%, P < 0.001) and in the OA (+19%, P = 0.003). Administration of L-NMMA alone reduced FPA (−21%, P < 0.001) and MFV in the MCA (−11%, P = 0.030) but did not change MFV in the OA. All hemodynamic effects of L-NMMA were reversed by L-arginine. However, neither L-NMMA nor L-arginine altered acetazolamide-induced changes in cerebral or ocular hemodynamic parameters. The present data indicate that acetazolamide-induced hemodynamic changes are not mediated by NO. Which mediators other than NO are involved in the hemodynamic effects as induced by carbonic anhydrase inhibitors remains to be elucidated.

Acetazolamide; cerebral blood flow; ocular blood flow; acidosis

IN OPHTHALMOLOGY, acetazolamide, a carbonic anhydrase inhibitor, is used in the treatment of primary and secondary open-angle glaucoma as well as in the acute treatment of angle closure glaucoma. In addition, acetazolamide has been suggested in the treatment of macular edema (2) and retinitis pigmentosa (1). A variety of studies indicate that acetazolamide induces cerebral (3, 4, 20) and ocular (5, 22) vasodilation in humans. The mechanism behind this vasodilator effect is still a matter of controversy. Intracellular and extra-

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Acetazolamide-induced cerebral and ocular vasodilation is still a matter of controversy. Intracellular and extracellular acidosis are assumed to play a major role in the potent cerebral vasoactive action of carbonic anhydrase inhibition. Several in vitro studies have shown that the carbonic anhydrase pharmacology and the L-arginine-nitric oxide (NO) pathway are closely related (19, 21). In addition, the cerebral vasodilator response to another stimulus, namely hypercapnia, which is assumed to induce an extracellular and intracellular fall in pH, is at least partially mediated by NO (10, 25, 32). We therefore investigated the role of NO in acetazolamide-induced vasodilation in ocular and cerebral vessels in humans. For this purpose the effect of acetazolamide on ocular and cerebral hemodynamic parameters was compared in the absence and in the presence of Nω-monomethyl-L-arginine (L-NMMA), a NO synthase inhibitor. We also investigated whether L-NMMA-induced hemodynamic changes are reversible by coinfusion of L-arginine, the precursor of NO.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of Vienna University School of Medicine. Twelve healthy volunteers were studied (age range, 22–31 yr; mean ± SD, 26.2 ± 3.3 yr). The nature of the study was explained, and all subjects gave written consent to participate. Each subject passed a screening examination that included medical history and physical examination, 12-lead electrocardiogram, complete blood count, activated partial thromboplastin time, prothrombin time, fibrinogen, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, total protein), hepatitis A, B, C, and HIV serology, urine analysis, and a urine drug screen. Subjects were excluded if any abnormality was found as part of the screening unless the investigators considered an abnormality clinically irrelevant. Furthermore, an ophthalmic examination, including slit-lamp biomicroscopy and indirect funduscopy, was performed on each subject before the study day. Inclusion criteria were normal ophthalmic findings and a refractive error of less than three diopters in either of both eyes.

Study Design

Subjects were studied in a placebo-controlled, randomized, double-blind, three-way crossover design with respect to L-NMMA, and in a placebo-controlled, randomized, double-blind design in two parallel groups with respect to L-arginine. A time schedule is given in Fig. 1. Washout periods of at least 5 days between study days were scheduled. Subjects abstained from alcohol and beverages containing xanthine derivatives for 12 h before drug administration. On the trial days subjects arrived after an overnight fast. After resting for...
at least 20 min in a sitting position to establish stable hemodynamic conditions, baseline measurements of hemodynamics, intraocular pressure (IOP), and exhaled NO were taken. Thereafter placebo or L-NMMA (bolus of 3 mg/kg for 5 min followed by 30 μg·kg⁻¹·min⁻¹ for 95 min or bolus L-NMMA of 6 mg/kg for 5 min followed by 60 μg·kg⁻¹·min⁻¹ for 95 min; Clinalfa, Läufelfingen, Switzerland) was administered intravenously. To maintain double-blind conditions, two syringes containing physiological saline solution were prepared and infused sequentially. Twenty minutes later, infusion of L-arginine or placebo was started according to randomization. Forty minutes after the start of L-NMMA or placebo infusions, acetazolamide (1,000 mg iv; Wyeth Lederle, Vienna, Austria) was administered for 5 min. Measurements were performed every 10 min after the start of the L-NMMA infusion and every 15 min after administration of acetazolamide. Subjects crossed over to the other treatments on the other trial days.

Study Methods

Blood pressure and pulse rate. Systolic and diastolic blood pressures (SBP and DBP, respectively) and mean arterial blood pressure were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded from a finger pulse-oxymetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Blood pressure were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Fundus pulsations. Pulse-synchronous pulsations of the eye fundus were assessed by laser interferometry on the subject's right eye. The method is described in detail by Schmetterer et al. (27). Briefly, the eye is illuminated by the beam of a single-mode laser diode with a wavelength (λ) of 783 nm. The light is reflected at both the front side of the cornea and the retina. The two reemitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. Distance changes between cornea and retina lead to a corresponding variation of the interference order (ΔN(t)). This change in interference order can be evaluated by counting the fringes moving inward and outward during the cardiac cycle. Changes in optical distance (ΔL(t)), corresponding to the cornea-retina distance changes, can then be calculated by ΔL(t) = ΔN(t)·λ/2. The maximum distance change is called the fundus pulsation amplitude (FPA), which estimates the local pulsatile blood flow (23). FPA was measured as the mean of at least five cardiac cycles. The short-term and day-to-day variability of the method is small, which allows detection of even small changes in local pulsatile blood flow after pharmacological stimulation (28). In contrast to systems recording ocular pressure pulse (8, 16), information on the ocular circulation can be obtained with high transverse resolution. To obtain information on the choroidal blood flow, the macula, where the retina lacks vasculature, was chosen for measurements.

Doppler sonography. Mean blood flow velocity (MFV), peak systolic flow velocity, and end-diastolic flow velocity were determined in the right ophthalmic artery (OA) with color Doppler ultrasound (9) and in the middle cerebral artery (MCA) with transcranial ultrasound. MFV was measured manually as time mean of the spectral outline. Measurements were performed with a 7.5-MHz probe in the OA and a 2-MHz probe in the MCA (CFM 750; Vingmed Sound, Horten, Norway). The OA was measured anteriorly at the point where it crosses the optic nerve. The sample volume marker was placed ~25 mm posterior to the globe. All parameters were determined as mean values over at least three cardiac cycles.

Exhaled NO was measured with a chemiluminescence detector (nitrogen oxides analyzer, model 8840; Monitor Labs) connected to a strip-chart recorder. The instrument was calibrated with certified gases (300 parts/billion NO in N₂; AGA, Vienna, Austria) diluted by precision flowmeters. A baseline signal was obtained with pure nitrogen. Subjects were instructed to fully inflate their lungs, hold their breath for 10 s, and exhale for 10 s into a Teflon tube; 1,000 ml/min of the exhaled air was allowed to enter the inlet port. Three consecutive readings were made at each measurement point under nasal occlusion. The end-expiratory values from the strip recorder readings were used for analysis to ensure that inspired NO from the ambient air did not alter the results (12).

IOP measurements. A slit lamp-mounted Goldmann applanation tonometer was used to measure IOP. Before each measurement, one drop of 0.4% benoxinate hydrochloride

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Fig. 1. Time schedule for 3 study days showing administration of N⁶-monomethyl-L-arginine (L-NMMA; dosage 1, 3.0 mg/kg for 5 min followed by 30 μg·kg⁻¹·min⁻¹ for 95 min; dosage 2, 6.0 mg/kg for 5 min followed by 60 μg·kg⁻¹·min⁻¹ for 95 min) or placebo, L-arginine (5 mg·kg⁻¹·min⁻¹ over 80 min) or placebo, and acetazolamide (1,000 mg over 5 min). Blood pressure was measured in 5-min intervals throughout study period. Pulse rate and electrocardiogram were measured continuously.
Data Analysis

All statistical analyses were done using the Statistica software package (release 4.5; StatSoft, Tulsa, OK). Changes in hemodynamic parameters were analyzed with repeated-measures ANOVA with the absolute values of the hemodynamic data. For data description, the effect of L-NMMA, L-arginine, and acetazolamide was expressed as percent change of the preceding values. The percent changes induced by acetazolamide were compared during the administration of L-NMMA and L-arginine together and are presented as means ± SE. A two-tailed P < 0.05 was considered the level of significance.

RESULTS

Baseline ocular hemodynamic parameters are presented in Table 1. There were no significant differences between the three study days at baseline.

Effects of Acetazolamide

As expected, acetazolamide caused a significant drop in IOP (Fig. 2; −29%, P < 0.001). This was accompanied by an increase in FPA (Fig. 2; +27%, P < 0.001) and a less pronounced increase in MFV in the OA (Fig. 3; +19%, P = 0.003). The effect in the MCA was stronger with an increase in MFV of +38% (Fig. 4; P < 0.001). No significant change in exhaled NO could be detected (Table 2). In contrast to ocular and cerebral hemodynamic parameters, systemic blood pressure and pulse rate showed only minor changes during administration of the carbonic anhydrase inhibitor (Table 2).

Effects of L-Arginine and L-NMMA

FPA was reduced during administration of the NO synthase inhibitor (Fig. 2; −21% and −15% for 6 mg/kg followed by 60 µg·kg⁻¹·min⁻¹ and for 3 mg/kg followed by 30 µg·kg⁻¹·min⁻¹, respectively, P < 0.001). MFV in the MCA showed a significant decrease (Fig. 4; −11%, P = 0.009) at the higher dosage of L-NMMA only. MFV in OA showed a tendency to decrease (Fig. 3). L-Arginine reverted all cerebral and ocular hemodynamic changes induced by both doses of L-NMMA. Immediately before the onset of the acetazolamide infusion, all cerebral and ocular hemodynamic parameters had returned to baseline values. DBP showed a significant increase during L-NMMA infusion (Table 2; higher dose: +26%, lower dose, +16%; P = 0.019), which was also antagonized by L-arginine (Table 3). In contrast, there was no significant change from baseline in SBP at both dosages of the NO synthase inhibitor (Table 2). A dose-dependent effect of L-NMMA was, however, observed on pulse rate (Table 2; higher dose, −16%; lower dose, −12%; P = 0.003). As expected, L-NMMA significantly reduced

Table 1. Baseline ocular hemodynamic parameters of 3 study days

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<th>Day 1</th>
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<th>Day 3</th>
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<tr>
<td>FPA, µm</td>
<td>4.1 ± 1.3</td>
<td>4.0 ± 1.4</td>
<td>4.1 ± 1.3</td>
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<tr>
<td>OA</td>
<td>0.84 ± 0.02</td>
<td>0.83 ± 0.03</td>
<td>0.83 ± 0.02</td>
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<tr>
<td>MFV, cm/s</td>
<td>19.0 ± 2.3</td>
<td>19.4 ± 2.4</td>
<td>20.4 ± 3.3</td>
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<tr>
<td>MCA</td>
<td>0.65 ± 0.03</td>
<td>0.64 ± 0.03</td>
<td>0.64 ± 0.03</td>
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<tr>
<td>MFV, cm/s</td>
<td>43.4 ± 6.7</td>
<td>43.9 ± 5.7</td>
<td>42.7 ± 9.1</td>
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<tr>
<td>IOP, mmHg</td>
<td>13.2 ± 1.0</td>
<td>12.6 ± 1.6</td>
<td>13.0 ± 1.5</td>
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All data are means ± SE; n = 12 subjects. FPA, fundus pulsation amplitude; OA, ophthalmic artery; MCA, middle cerebral artery; RI, resistive index; MFV, mean blood flow velocity; IOP, intraocular pressure.
Acetazolamide-induced changes in FPA during baseline conditions (+27%) were not affected by L-NMMA (+29% for both doses), L-arginine alone (+23%), or L-arginine and L-NMMA coinfusion (+22% and +28% for lower and higher dose, respectively; Fig. 2). Acetazolamide-induced change in MFV in the MCA (+38% at baseline) did not show any significant difference during administration of L-NMMA (+37% for both doses) or L-arginine (+37%) or during coinfusion of L-NMMA and L-arginine (+33% for both doses of L-NMMA, Fig. 4). Neither L-NMMA nor L-arginine significantly altered acetazolamide-induced changes in any other cerebral or ocular hemodynamic parameter or in IOP (data not shown).

**DISCUSSION**

As expected, acetazolamide induced strong vasodilation in ocular and cerebral vessels in the present study. This effect was comparable during placebo and L-NMMA administration, which indicates that acetazolamide-induced vasodilation is not NO dependent. L-NMMA, however, dose-dependently decreased blood flow velocity in the MCA and ocular FPA. Because these effects were reversible by L-arginine, the precursor of NO synthesis, our results indicate that NO contributes to vascular tone in the MCA and the choroid.

Several observations indicate that in the present study L-NMMA inhibited NO synthase to a significant degree. In previous studies comparable doses of L-NMMA were sufficient to blunt the hemodynamic effects of hypercapnia (25), insulin (24), and histamine (29) and reduced the concentration of NO in exhaled air.
The present study also observed a decrease of exhaled NO after L-NMMA administration. Although exhaled NO is not an appropriate index of NO synthase inhibition at the level of ocular and cerebral circulation, it clearly demonstrates the efficacy of the L-NMMA as an inhibitor of NO synthase. Another line of evidence for partial NO synthase inhibition arises from the observation that all hemodynamic effects elicited by L-NMMA in the present study were reversible by coadministration of L-arginine. This is particularly important, because concerns regarding the specificity of L-NMMA as an inhibitor of NO synthase have been raised.

L-NMMA significantly reduced FPA in a dose-dependent manner in the present study. This may be caused by a particularly high reactivity of the choroidal vessels to changes in NO production related to the high perfusion rate in this vascular bed (26). In contrast to our previous findings (25), NO synthase inhibition reduced MFV in the MCA. This discrepancy may at least partially be caused by the higher dose we used in the present study. A fall of cerebral blood flow after high-dose L-NMMA (10 mg/kg bolus injection) was also observed by White et al. (33), but only in the internal and common carotid artery. Our findings are also in accordance with some earlier animal studies, which demonstrated a reduction of cerebral blood flow after administration of NO synthase inhibitors (6, 14, 30).

A previous study revealed that acetazolamide-induced vasodilation in rats is not dependent on NO (32). However, interspecies differences regarding this question may well exist, as they appear with the NO dependence of hypercapnia-induced vasodilation (10, 18, 25, 31–33). Hence we decided to perform the present human trial, although such an investigation is obviously limited to a noninvasive method for the assessment of ocular and cerebral hemodynamics.

Data on prolonged acetazolamide treatment indicate that the initial cerebral blood flow increase is caused by transient extracellular acidosis, whereas the long-term effects of acetazolamide result from intracellular acidosis (7). In contrast, hypercapnia causes extracellular as...
well as intracellular acidosis (11, 17). Whether this is related to differences in the NO dependence of the two stimuli remains to be established. Moreover, other hitherto unidentified mechanisms may contribute to vasodilation elicited by acetazolamide.

Several methodological limitations concerning our results obtained in the cerebral and ocular circulation have to be mentioned. Doppler sonography in the MCA and OA measures blood flow velocity in these vessels rather than blood flow. Because no data on vessel diameter were available from our measurements, extrapolation in terms of perfusion has to be done with caution. If acetazolamide also increased the diameter of the large arteries under study, our velocity measurements may underestimate the effect on blood flow. This limitation has been mentioned previously (13) for the MCA but also holds true for the OA. The reduction of MFV in MCA during administration of L-NMMA provides evidence that cerebral blood flow during NO synthase inhibition is decreased. If L-NMMA induced a change in vascular tone in the MCA or OA, the perfusion effect may again be underestimated by velocity measurements. Regarding FPA measurements, it has to be pointed out that only pulsatile blood flow is measured. This limitation has been discussed in detail in previous reports employing this technique for the assessment of ocular hemodynamics (15, 25). Again, the effect of peripheral vasoconstrictors and vasodilators on total choroidal blood flow is rather underestimated (25).

Acetazolamide-induced hemodynamic effects were not affected by NO inhibition. An effect of higher doses of L-NMMA on acetazolamide-induced vasodilation cannot be excluded but seems to be unlikely. In addition, a human trial with extensively higher doses of L-NMMA is hampered by ethical considerations. An additional limitation in the interpretation of acetazolamide-induced hemodynamic effects arises from the vasoconstrictor action of L-NMMA. Compared with placebo, L-NMMA altered vascular tone in cerebral and ocular vessels. Hence acetazolamide exerted its vasoactive effect on a preconstricted vessel. However, because the hemodynamic effects of NO synthase inhibition were small in this trial, this limitation seems to be of minor importance.

In conclusion, our study indicates that the vasodilator action of acetazolamide is not NO dependent. Hence there seems to be a considerable difference in the mechanisms behind ocular and cerebral vasodilation as elicited by hypercapnia or acetazolamide. Which mediators other than NO are involved in the hemodynamic effects induced by carbonic anhydrase inhibitors remains to be elucidated.

Perspectives

A variety of animal studies have been performed to elucidate the role of NO in the regulation of cerebral and ocular blood flow. However, only few data are available from human studies in vivo, which may be related to the difficulties in assessing cerebral and ocular blood flow. The present study is an attempt to characterize the role of NO in these vascular beds. Additional studies in humans, however, remain to be done before therapeutic regimens in ocular and cerebral vascular disease can be directed to the l-arginine-NO pathway.

Excellent technical assistance by Ursula Graselli is acknowledged. Address for reprint requests and other correspondence: L. Schmetterer, Dept. of Clinical Pharmacology, Währinger Gürtel 18–20, A-1090 Vienna, Austria (E-mail: leopold.schmetterer@univie.ac.at).

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