In Vivo Effects of Alpha-Adrenoceptor Agonists and Antagonists on Pial Veins of Cats

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SUMMARY  Cerebral blood volume and intracranial pressure may be modified by influences on cerebral veins. The known adrenergic innervation of cerebral veins and their sensitivity to norepinephrine raised the question, whether pial veins can be selectively influenced through adrenoceptors in vivo. Therefore, alpha1 and alpha2 adrenoceptor agonists and antagonists were locally injected into the perivascular space of pial veins using the microapplication technique. The alpha1 and alpha2 adrenoceptor antagonists, prazosin and yohimbine, had only minor effects on pial veins. Both antagonists blocked constrictions induced by norepinephrine (10^{-5}M) in a concentration dependent manner (10^{-7}-10^{-4}M). The alpha1 adrenoceptor agonist phenylephrine caused significant (10^{-7}-10^{-4}M) constriction of pial veins, with a maximum of 11.6% of initial diameter at 10^{-3}M. Oxymetazoline, an alpha2 receptor agonist, induced a significant constriction only at 10^{-3}M (5.1%). Since both alpha1 and alpha2 adrenoceptor agonists are less potent constrictors of pial veins than norepinephrine in vivo, a preferential use of alpha1 or alpha2 adrenoceptor agonists cannot be recommended from these experiments, if a therapeutic reduction of intracranial pressure or blood volume is desired.

WHEREAS THE TONE OF CEREBRAL ARTERIES and arterioles determines the level of blood flow to the different structures of the brain, the cerebral veins seem to be of importance for the regulation of the intracranial pressure and cerebral blood volume. Only recently interest has been focused on the mechanisms which may regulate the tone of cerebral veins. The sympathetic nervous system appears to influence the cerebral venous tone, since noradrenergic nerve fibers innervate pial veins down to 50 µm diameter and electrical stimulation of the cerebral sympathetic fibers constricts pial veins. This constriction is not caused by passive venous collapse as a consequence of a decreased intravascular pressure due to arterial constriction; rather it is an active process, since comparable constriction can be induced by local perivascular microapplication of norepinephrine to pial veins. The pial venous constriction during sympathetic stimulation appears to be mediated to a large extent by alpha-adrenoceptors, since the effect is abolished by phenoxybenzamine but not by pranoprolol. A more selective influence on pial venous alpha-adrenoceptors would be desirable in therapeutic attempts to alter intracranial pressure and cerebral blood volume. Therefore, the effects of drugs known as alpha1 and alpha2 adrenoceptor agonists and antagonists were tested in the present study. Historically, the first differentiation between pre- and postsynaptic receptors was made between pre- and postsynaptic receptors. After they were found to differ, they were called alpha1 and alpha2 adrenoceptors. After the discovery of post-synaptic alpha1 adrenoceptors we have to distinguish between pre- and postsynaptic alpha2 adrenoceptors.

Methods

Experiments were performed on 21 adult cats of either sex weighing between 2.1 kg to 3.4 kg. The cats were anesthetized with glucochloralose (40-50 mg/kg i.v.) and immobilized with pancuronium bromide (0.13 mg/kg body weight x hour i.v.). They were artificially ventilated via a tracheal cannula by a Bird Mark 8 respirator. The right femoral vein was cannulated for infusion of 2.5 ml/kg x h Tyrode’s solution. The right femoral artery was also cannulated for arterial blood gas analysis (AVL Gas Check 939, Graz, Austria) as well as for the continuous recording of arterial blood pressure, measured by a Statham strain gauge and Hellige transducer. The arterial pH was 7.31 ± 0.33, pCO2 37.6 ± 4.3 mmHg and pO2 109.4 ± 11.6 mmHg (k ± sd). Only cats with a mean arterial blood pressure of more than 100 mmHg were used for the experiments during which body temperature was maintained between 37°C and 38°C with a heating pad. The cats were placed in prone position with their heads firmly fixed in a stereotactic headholder. A craniotomy of 4–5 cm² was made in the temporo-parietal region. Without touching the brain the dura was carefully removed having covered the pial surface with a layer of 1–2 cm of warmed (37°C) paraffin oil.

Glass micropipets with sharpened tips (8–10 µm) were filled with the test solutions and mounted on a micromanipulator. Then their tip was positioned in the immediate vicinity of a pial vein.

By applying pressure to a syringe connected with the micropipet by plastic tubes, 1–3 µl of fluid were injected into the perivascular space. The following drugs were used: norepinephrine and phenylephrine were bought from Sigma, prazosin and oxymetazoline were supplied as a generosity from Pfizer and yohimbine was kindly supplied by Dr. L. Edvinsson, Lund, Sweden. The drugs were dissolved in artificial cerebrospinal fluid (CSF) with the following composition: Na⁺ 156 mM, K⁺ 3 mM, Ca²⁺ 1.5 mM, HCO₃⁻ 15 mM, Cl⁻ 147 mM, pH 7.28. The mock spinal fluid was prepared for each experiment and perfused with 95% N₂ and 5% CO₂ (equilibrated in water) to prevent autoxidation and to assure full equilibration of the mock CSF with CO₂. To obtain the mock CSF containing prazosin, prazosin was first dissolved in 20% lactic
acid, then diluted by a factor of 500 with the mock CSF and then titrated back by adding the corresponding amount of NaOH. The micropipets were filled immediately before the perivascular injection, which lasted 1 minute. Photographs were taken through a Wild-Heerbrugg stereozoom microscope equipped with a Leitz photographic camera before and 20, 40 and 60 seconds after the injection of the test solutions. The pial veins had a resting diameter ranging from 61 to 816 µm (mean 257 µm). To obtain general information about the reactivity of the pial vessels, the reactions of pial arteries to perivascular microinjections of acidic and alkaline mock CSF were tested before and during each experiment. The pial arteries constricted to alkaline solutions and dilated to acidic ones in the typical way that has been shown previously. The responses of the veins to topical application of the different drugs were calculated as a percentage of resting diameters before injection of the mock CSF. For the statistical analysis of the data the vascular responses to the solvent solution (15 mM HCO₃⁻) were compared with the vascular responses during application of the solvent solution which contained the respective blocking or stimulating substance. The responses to the different concentrations were compared by the nonparametric Wilcoxon Matched Pairs Signed Ranks test. An overall value of p < 0.05 was chosen as level of significance according to the procedure established by Bonferroni-Holm for multiple statistical testing.

Results

The effects of the alpha₁ and alpha₂ adrenoceptor agonists given in ascending concentrations are shown in figure 1. Both the alpha₁ adrenoceptor agonist phenylephrine and the alpha₂ adrenoceptor agonist oxymetazoline induced constriction of pial veins. The constriction was statistically significant for concentrations of phenylephrine ranging from 10⁻⁷ to 10⁻³ M, but only at 10⁻³ M oxymetazoline. Local perivascular application of the alpha₁ adrenoceptor antagonist prazosin and the alpha₂ adrenoceptor antagonist yohimbine did not yield significant venous responses as shown in figure 2.

The participation of alpha₁ and alpha₂ receptors in the norepinephrine-induced constriction of pial veins was tested by application of norepinephrine together with the respective blocking substances. For this purpose ascending concentrations of either prazosin or yohimbine were applied in mock CSF that also contained norepinephrine (10⁻⁵ M). This experimental approach is only justified if repeated applications of norepinephrine induce reproducible constriction of pial veins. Figure 3 shows that this prerequisite was fulfilled. The constriction induced by norepinephrine was always significantly different from the venous response to the previous application of the solvent alone. The effects of ascending concentrations of the alpha₁, adrenoceptor antagonist prazosin and the alpha₂ adrenoceptor antagonist yohimbine on the norepinephrine-induced constriction are shown in figure 4. Norepinephrine and the respective antagonist were applied simultaneously in the same microinjection pipet. The constrictor effect of norepinephrine was blocked by prazosin and yohimbine. The reduction of the norepinephrine-induced constriction was significant at 10⁻⁷ M prazosin and at 10⁻⁷ M yohimbine and at all higher concentrations of the blocking agents. At their highest concentrations, 10⁻⁴ M, both antagonists abolished the norepinephrine-induced constriction.

Discussion

It was the aim of the present study to test whether substances known to activate or block alpha₁ or alpha₂ adrenoceptors selectively in other organs and vessels may be effective in reducing pial venous diameter in vivo. Such an effect may be helpful with respect to a reduction of cerebral blood volume or intracranial pressure. The microapplication method employed in the present study allows the detection of direct drug effects on vessels under in vivo conditions. The results show that the alpha₁ and alpha₂ adrenoceptor agonists phenylephrine and oxymetazoline are both able to reduce pial venous diameter in vivo; however, their effects are weak when compared to that of norepinephrine. A complete characterization of pharmacological receptors was not intended in the present study; it is possible only in isolated vessel preparations, because equilibrium conditions are not likely to exist during the application time of the drugs of one minute.

The presence of both alpha₁ and alpha₂ adrenoceptors has been postulated in isolated femoral and saphe-
nous veins. With respect to cerebral vessels mainly arteries have been analyzed for the existence of alpha_1 and alpha_2 adrenoceptors. An extreme variability of the results has been found, depending on the species investigated, as has been summarized recently. An unusual type of adrenoceptor may exist at isolated pial and basilar arteries. In the isolated feline middle cerebral and bovine pial artery, the postsynaptic alpha-adrenoceptor seems to be mainly of the alpha_2-type. Experiments on isolated pial veins of cats seem difficult to perform because of the weak tension developed by these veins. Tension development is high enough in isolated pial veins of goats. In these vessels a blocking action of phentolamine on norepinephrine-induced constrictions has been demonstrated. A subclassification of the adrenoceptors was not performed in these studies.

The effects measured in the present experiments appear to be mediated mainly by postsynaptic receptors. Among the tested substances presynaptic effects could be expected mainly from yohimbine. An effect of yohimbine on cerebral blood flow has been verified under conditions of cervical sympathetic stimulation. Under these conditions, a reduction of the cerebral blood flow was detected on the side of stimulation after pretreatment with yohimbine. This reduction of blood flow can be ascribed to an increased concentration of norepinephrine at the cerebral resistance vessels after the blockade of the presynaptic receptors, which inhibit the release of norepinephrine. Yohimbine did not affect the cerebral blood flow under control conditions without sympathetic stimulation, indicating a low rate of feedback inhibition of norepinephrine release during control conditions. These results of blood flow measurements are in agreement with the present study of pial veins: local perivascular application of yohimbine did not induce a significant change in venous diameter, thus indicating a minor importance of presynaptic mechanisms for the resting venous diameter. A blockade of a supposed presynap-

**Figure 2.** Concentration response curves for the alpha_1 receptor antagonist prazosin (upper part) and the alpha_2 antagonist yohimbine (lower part). x ± SD. n = number of vessels tested.

**Figure 3.** Effect of alternating application of the solvent solution (15 mM HCO_3^-; open symbols) and the norepinephrine containing solvent (10^-5 M NE, closed symbols). Repeated application of norepinephrine induces a comparable degree of vascular constriction. x ± SD. n = number of vessels tested.

**Figure 4.** Effects of the alpha_1 receptor antagonist prazosin (upper part) and the alpha_2 receptor antagonist yohimbine (lower part) on norepinephrine-induced constrictions (10^-5 M NE). First, norepinephrine (10^-5 M) was given alone. Then, the lowest concentration of the respective blocking agent was given alone in order to quantify the effect of the antagonist under these conditions. This was followed by the application of the same concentration of the antagonist together with norepinephrine (10^-5 M). This protocol was repeated for the next higher concentration of the antagonist and for the combination of norepinephrine and the antagonist etc. x ± SD. n = number of vessels tested.
tic inhibition of norepinephrine release would result in an increased perivascular concentration of norepinephrine. A vasoconstriction of the pial veins would then be expected because of the constrictor effect of norepinephrine on pial veins. 2,4 The small non-significant reduction in pial venous diameter during yohimbine application shown in figure 2 (−3.1% at 10⁻³ M yohimbine), if completely ascribed to an enhanced release of norepinephrine, would only correspond to a concentration of 4 × 10⁻⁸ M norepinephrine. After application of norepinephrine, yohimbine alone induced a stronger constriction (figure 4). This constriction was due to a persistence of norepinephrine effects only in these experiments. It is not clear whether this persisting effect of norepinephrine had anything to do with the application of yohimbine in these experiments. A predominant postsynaptic action of yohimbine is supported by the fact that the constrictor effect of exogenous norepinephrine can be reduced by yohimbine (fig. 4 lower part), which can be explained reasonably only by a blocking action on postsynaptic receptors. Concerning the alpha, adrenoceptor antagonist prazosin, an action on postsynaptic receptors is generally accepted.3,25

Whereas a postsynaptic action of the alpha₁ and alpha₂ adrenoceptor antagonists is very likely in the present experiments, their specificity cannot be verified using the in vivo approach. As shown in figure 4, each antagonist is able to completely block the constrictor effect of norepinephrine if given in high concentrations. The results of the application of alpha₁ and alpha₂ adrenoceptor agonists show a significant constriction for a wide range of phenylephrine concentrations but only for the highest concentration of oxymetazoline (10⁻³ M). The large scatter of venous reactions during oxymetazoline precluded a significance of constriction at lower concentrations. The constriction induced by both adrenoceptor agonists is not sufficient to account quantitatively for the constriction found after application of comparable concentrations of norepinephrine. Whereas norepinephrine (10⁻³ M) induced a vеноconstriction of 18.9%,4 the constriction at the same concentration of phenylephrine was only 6.1% and of oxymetazoline 5.3% (fig. 1). The alpha₁ and alpha₂ adrenoceptor agonists appear to exert their effects on the pial veins mainly through the postsynaptic receptors in analogy to the antagonists. This can be concluded from the fact that the most likely candidate for a presynaptic action used, the alpha₁, adrenoceptor agonist oxymetazoline,3,4 exerts no significant effect on venous diameter over a wide range of concentrations (fig. 1) thus making any significant influence of presynaptic mechanisms in the present experiments unlikely. This is in accordance with the conclusion drawn for the effects of yohimbine earlier in this discussion. Under different experimental conditions with an increased transmitter release, a presynaptic action of the alpha₁ agonist clonidine has been verified for pial arteries in vivo; the constriction of pial arteries exerted by bilateral stimulation of the cervical sympathetic chains was reduced by i.v. clonidine.26

Whereas these other studies have shown that presynaptic effects of alpha₁ adrenoceptor blocking and stimulating substances can be disclosed under special conditions in arteries, the present investigation demonstrates mainly postsynaptic effects in veins. Since both alpha₁ and alpha₂ adrenoceptor agonists are less potent constrictors of pial veins than norepinephrine in vivo, the present experiments do not support preferential use of alpha₁ or alpha₂ adrenoceptor agonists, if a reduction of intracranial pressure or blood volume is desired.

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