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

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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 diameter1-2 and electrical stimulation of the cervical sympathetic fibers constricts pial veins.3 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.3-4 The pial venous constriction during sympathetic stimulation appears to be mediated to a large extent by alpha-adrenoceptors, since the effect is abolished by phentolamine but not by propranolol.1 A more selective influence on pial venous alpha-adrenoceptors would be desirable in therapeutic attempts to alter intracranial pressure and cerebral blood volume. Therefore, since alpha and alpha2 adrenoceptor agonists and antagonists were tested in the present study. Historically, the first differentiation was made between pre- and postsynaptic receptors. After they were found to differ, they were called alpha1 and alpha2 adrenoceptors. After the discovery of postsynaptic alpha-adrenoceptors we have to distinguish between pre- and postsynaptic alpha2 adrenoceptors.5-9

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 × 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 × 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 (± 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 autodiation 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.
Results

The effects of the alpha, and alpha2 adrenoceptor agonists given in ascending concentrations are shown in figure 1. Both the alpha, adrenoceptor agonist phenylephrine and the alpha, adrenoceptor agonist oxymetazoline induced constriction of pial veins. 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 alpha, 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 alpha, 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.12

The presence of both alpha, and alpha2 adrenoceptors has been postulated in isolated femoral and saphe-
nous veins.\textsuperscript{13} With respect to cerebral vessels mainly arteries have been analyzed for the existence of alpha\textsubscript{1} and alpha\textsubscript{2} adrenoceptors. An extreme variability of the results has been found, depending on the species investigated, as has been summarized recently.\textsuperscript{14} An unusual type of adrenoceptor may exist at isolated pial\textsuperscript{15} and basilar arteries.\textsuperscript{16,17} In the isolated feline middle cerebral\textsuperscript{18} and bovine pial\textsuperscript{19} artery, the postsynaptic alpha-adrenoceptor seems to be mainly of the alpha\textsubscript{2}-type. Experiments on isolated pial veins of cats seem difficult to perform because of the weak tension developed by these veins.\textsuperscript{20} 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.\textsuperscript{21} 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.\textsuperscript{6,22} 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.\textsuperscript{23} 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.\textsuperscript{24} 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-
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 \(10^{-5}\) M yohimbine), if completely ascribed to an enhanced release of norepinephrine, would only correspond to a concentration of \(4 \times 10^{-8}\) 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 alpha, and alpha, 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 alpha, and alpha, adrenoceptor agonists show a significant constriction for a wide range of phenylephrine concentrations but only for the highest concentration of oxymetazoline \((10^{-3} \text{ M})\). 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 \((10^{-3} \text{ M})\) induced a venoconstriction of 18.9%,\(^4\) the constriction at the same concentration of phenylephrine was only 6.1% and of oxymetazoline 5.3% (fig. 1).

The alpha, and alpha, 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 alpha, adrenoceptor agonist oxymetazoline,\(^7\) 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 alpha, 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 alpha, adrenoceptor blocking and stimulating substances can be disclosed under special conditions in arteries, the present investigation demonstrates mainly postsynaptic effects in veins. Since both alpha, and alpha, adrenoceptor agonists are less potent constrictors of pial veins than norepinephrine in vivo, the present experiments do not support preferential use of alpha, or alpha, adrenoceptor agonists, if a reduction of intracranial pressure or blood volume is desired.

**Acknowledgment**

The authors thank Prof. Dr. L. M. Auer, Graz, Austria, and Dr. L. Edvinsson, Lund, Sweden, for their valuable suggestions concerning the experiments.

**References**

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Stroke. 1985;16:880-884
doi: 10.1161/01.STR.16.5.880

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