Direct Evidence for Absence of Beta-Adrenergic Receptors in Rat Cerebral Vessels

Histochemical Study with a Fluorescent Beta-Blocker

ELDAD MELAMED, M.D., DAPHNE ATLAS, PH.D., AND MOSHE LAHAV, M.D.

SUMMARY
A fluorescent marker for beta-adrenergic receptor sites, 9-amino-acridin propranolol (9-AAP), was administered intravenously to rats. In contrast to other tissues which are known to contain beta-adrenergic receptors, 9-AAP fluorescence was not observed in the walls of the pial as well as parenchymal cerebral vessels. These negative findings strongly suggest that in the rat, beta-adrenergic receptors are not present in the cerebral vasculature. The role of the alpha-adrenergic receptors needs more study.

CONSIDERABLE EVIDENCE has demonstrated that the mammalian cerebral arteries and arterioles are innervated by an extensive network of norepinephrine-containing nerve terminals. An abundant literature has dealt with the long and yet unsolved dispute concerning the role of the sympathetic nervous system in the control and regulation of the cerebral circulation. However, little information was available about the type of the post-synaptic receptors which mediate the effects of norepinephrine in the cerebral arterial system.

The methods which have been used for the identification of alpha- and beta-adrenergic receptors in various tissues are largely indirect. These are based on the evaluation of the potency order of different catecholamines in producing certain pharmaco-physiological effects and on the ability of specific blocking agents to antagonize these responses. Similar approaches have been recently employed in the search for cerebrovascular beta-adrenergic receptors.

Several studies in humans and in experimental animals have shown that the potent beta-adrenergic antagonist propranolol reduces cerebral blood flow (CBF). These findings were interpreted as evidence for the presence of beta-receptors in the cerebral vessels. On the other hand, other authors reported that no significant CBF changes had occurred either following administration of propranolol, or of the classical beta-receptor agonist isoproterenol. Propranolol can reduce the cerebral metabolic rate for both oxygen and glucose. It may, therefore, be suggested that propranolol decreases CBF not by a primary direct effect on cerebrovascular beta-receptors but secondarily, through reduction of cerebral metabolism. In addition, propranolol may induce bradycardia and arterial hypotension through a direct effect on the heart or by a specific action on cerebral cardiovascular centers. These effects may further contribute to the observed reduction in CBF after administration of propranolol.

A study of canine cerebral vessels suggested beta-adrenergic receptor mediated changes in cerebral vascular resistance. Edvinsson and Owman have demonstrated blockade of isoproterenol-induced constrictions of cerebral arteries in vitro by propranolol and cited this as further evidence for the presence of beta-receptors. In contrast, Rosenblum and Kuschinsky and Wahl could not show significant alterations in pial arterial diameter by either isoproterenol or propranolol. They suggested a low functional significance of beta-receptors and absence of beta-mediated tone changes in the cerebral vessels.

All of these contradictory findings may be partially due to the variety of methods and animal species used in these studies. The resultant conflicting interpretations concerning the presence or absence of beta-adrenergic receptors in the cerebral vessels, indicate the need for a more direct approach.

We have recently developed a new histochemical fluorescence method for the direct detection and localization of beta-adrenergic receptors. This is based on the in vivo administration of 9-amino-acridin propranolol (9-AAP) which is a fluorescent potent beta-adrenergic antagonist. The material 9-AAP has been used to label beta-adrenergic receptors in the rat cerebellum, spinal cord, cerebral cortex, myocardium, and kidney. In the present study this compound was used in an attempt to detect beta-adrenergic receptors in the cerebral vessels of the rat.

Methods
The material 9-AAP is a fluorescent analogue of propranolol. Its chemical structure is (N-[2-hydroxy-3-naphthoxypropyl]-N'-[9-amino acridin]isopropylamine) and its spectroscopic molar extinction coefficient, Σ∞ in water, is $1.07 \times 10^3$.

Albino rats of either sex, weighing 180-250 g were used in this study. The 9-AAP was dissolved in sodium phosphate buffer, 0.02M, pH 7.4 and a dose of 5 mg/kg body weight was administered by slow injection into the tail vein. The animals were decapitated under light ether anesthesia five, ten, or twenty minutes after 9-AAP injection. The brains were quickly removed, embedded in "tissue OCT compound" (Ames) and frozen in liquid nitrogen. Later, 6-8 μm coronal, sagittal and horizontal sections were cut in a cryostat at $-20°C$. The frozen sections were mounted on glass slides, air dried, and covered by sodium phosphate buffer, 0.08M, pH 7.4. The sections were studied under phase contrast and transmitted ultraviolet illumination on a Zeiss Universal Fluorescence Microscope with HBO 200-W.
Results

In the brains, intense yellow dotted 9-AAP fluorescence distinctly labeled the Purkinje cell layer of the cerebellar cortex, as well as the hippocampal pyramidal cell layer, the granule cell layer of the dentate gyrus, the pyramidal cell layer of the piriform cortex (fig. 1) and the basal layers of the neocortex, as already reported.26-28 By contrast, the walls of the pial arteries (large, medium-sized and small) and arterioles, were completely devoid of 9-AAP fluorescence in all animals (figs. 1-3). Likewise, 9-AAP fluorescence was absent from cerebral parenchymal blood vessels. The only fluorescence which was observed in the cerebral vasculature was the familiar nonspecific bluish fluorescence within the internal elastic laminae.

Discussion

Previous studies with 9-AAP have shown that it may be used effectively as a fluorescent probe for the direct histochemical identification and localization of beta-adrenergic receptor sites.26-28 In the present study, 9-AAP fluorescence was not observed in cerebral vessels of the rat. Within the cerebral vasculature the pial vessels are the most extensively innervated by the sympathetic nervous system. The density of the noradrenergic innervation gradually decreases from the proximal to the distal parts of the cerebral vascular tree.1, 4, 8 It was demonstrated that the sympathetic axon terminals make synaptic contacts with muscle cells in the media layer of the cerebral arterial wall.5, 34 These data would therefore make the media layer of pial vessels the most likely site for a higher concentration of the beta-adrenergic receptors, if they are indeed present in the cerebral vessels. However, even there, fluorescent 9-AAP binding sites were not observed.

The absence of 9-AAP fluorescence strongly suggests that beta-adrenergic receptors are not present in the walls of the rat cerebral arterial system. An alternative possibility is that the number of beta-adrenergic receptor sites in the cerebral vessels is too low to be detected by 9-AAP labeling. Either way, our findings indicate that in the rat, the beta-adrenergic receptors have little if any role in the neurogenic control and regulation of the cerebral circulation. This is in accordance with the results of other investigations.16, 17, 20, 21 It is therefore feasible that only the alpha-adrenergic receptors are involved in the mediation of the effects of norepinephrine in the cerebral vessels. There is evidence to support this view,21, 22, 30-33 but this too remains to be further substantiated by more direct approaches.

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References

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E Melamed, D Atlas and M Lahav

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