EFFECT OF CALCIUM BLOCKERS ON THE MORPHOLOGICAL AND PHYSIOLOGICAL STATE OF THE CEREBRAL CORTICAL CAPILLARY SYSTEM

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A leading place among substances which can be used to treat essential hypertension and coronary disease, as well as cardiac arrhythmias, is occupied by calcium antagonists, the principal mechanism of action of which is their ability to block calcium channels and to suppress the transmembrane Ca^{++} current. It has been suggested that many smooth muscle relaxants, including nearly all the coronary dilators, exert their action through inhibition of the Ca^{++} contractile mechanism [7]. Meanwhile blockers of calcium channels inhibit noradrenalin (NA) release from central and peripheral noradrenergic neurons [5]. At least two mechanisms thus participate in their action: relaxation of smooth-muscle cells and inhibition of NA secretion, both of which are highly essential for the formation of vascular tone.

The aim of this investigation was to study changes in the morphological and physiological state of the capillary system of the cerebral cortex after blocking of the transmembrane Ca^{++} current by its antagonists through the exogenous and endogenous effect of NA.

EXPERIMENTAL METHOD

Experiments were carried out on 40 cats anesthetized with pentobarbital (25 mg/kg). Verapamil (from LEK, Yugoslavia) was injected into the carotid artery in a dose of 0.4 mg/ml. Nifedipine (Corinfar, from Germed, East Germany) was injected in a dose of 5 mg/ml/min, noradrenalin (from Lederle, USA) in doses of 10 and 1 μg/ml/min, and clonidine (clofelin) and phenylephrine (mezaton) were injected in doses of 0.5 mg/ml/min into the carotid artery in the course of 3 min. Material for morphological investigation was taken through a burr-hole drilled in the frontal part of the skull, fixed in formalin, then treated by a noninjection method to demonstrate the intracerebral microcirculatory sphincters, based on direct staining of the vessel walls. The diameter of the capillaries and the number of greatly constricted capillaries (GCC) in 100 fields of vision were determined on microscopic preparations. The significance of differences between data for the experimental and control groups was estimated by the Fisher–Student test.

EXPERIMENTAL RESULTS

Intracarotid injection of verapamil led to a definite decrease in the number of nonfunctioning capillaries (by 63.4%) and to hardly visible constriction of the capillary lumen (by 6.8%) compared with the control. The effect of nifedrine on the number of GCC (a decrease by 16.7%) was much weaker than that of verapamil. Some increase in the capillary lumen also was observed (Fig. 1). The effects described above are evidence that the drugs tested exert their action mainly in the region of the precapillary sphincters, for we know that an increase or decrease in the number of nonfunctioning capillaries is mainly controlled by the precapillary sphincters [1]. The ability of verapamil to increase the number of actively functioning capillaries in the cerebral cortex by a much greater degree than nifedrine also is evidence that a more active role in the mechanisms of change of basal tone of the precapillary sphincters is evidently played by the gating mechanisms of the Ca channels than by the slow inward flow of calcium, on which 1,3-dihydropyridines (nifedrine, etc.) mainly act. The basis for this assumption is provided by data showing that verapamil acts on the "internal gate" of the Ca-channels of cell

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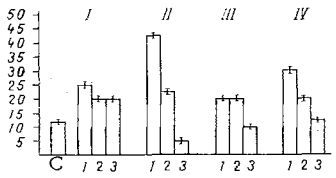

Fig. 1

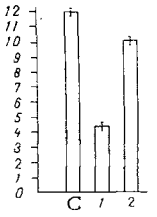

Fig. 2

Fig. 1. Effect of verapamil (1) and nifedrine (2) on number of GCC in cerebral cortex. Here and in Fig. 2: C) control.

Fig. 2. Effect of calcium antagonists on number of nonfunctioning capillaries. I: 1) NA, 2) verapamil + NA, 3) nifedrine + NA. II: 1) Phenylephrine, 2) verapamil + phenylephrine, 3) nifedine + phenylephrine. III: 1) Clonidine, 2) verapamil + clonidine, 3) nifedrine + clonidine. IV: 1) SNS, 2) verapamil + SNS, 3) nifedrine + SNS.

membranes, and the strength of its action, moreover, largely depends on the state of the gate itself. If it is open the effect of verapamil is exhibited to the full [4]. Unlike verapamil, nifedrine acts mainly on the "external gate" of the Ca-channels, where receptors binding bivalent cations and blocking the inward slow calcium current are located, without involving "gating mechanisms" of the Ca-channels [4]. Under adrenergic loading conditions the response of the precapillary sphincters to the action of calcium antagonists is substantially altered. The first point to note is that under the influence of NA (an α_1/α_2-agonist), phenylephrine (α_1-agonist), and clonidine (α_2-agonist) considerable changes take place in the morphological and functional state of the cerebral cortical capillary system. For instance, in response to intracarotid infusion of NA the number of GCC increased by 108%. Under the influence of phenylephrine this effect reached 254%, but with clonidine the effect was only 66%. The diameter of the capillaries also was reduced somewhat (Fig. 2), more especially by phenylephrine (27.3%). According to the strength of their action on precapillary sphincters, the test substances could thus be arranged in the following order: phenylephrine → NA → clonidine. When the observed effects are evaluated the following point must be noted. Besides presynaptic α_2- and postsynaptic α_1-adrenoreceptors, blood vessels also contain postsynaptic α_2-receptors, located mainly extrasynaptically, nearer to the intima in the inner layers of the media, by contrast with α_1-receptors, which are located mainly on the adventitial-medial boundary in the immediate vicinity of nerve endings [6]. It is considered that α_1-adrenoreceptors are protected against the influence of exogenous NA, brought by the blood stream, chiefly through functioning of the neuronal uptake mechanisms, and that vasoconstrictor responses to sympathetic nerve stimulation are effected mainly through α_1-receptors. Effects of exogenous NA, on the other hand, are realized through α_2-adrenoreceptors. Since the distribution and density of the noradrenergic innervation of the blood vessels differs depending on the vascular region, the relations between postsynaptic α_1- and α_2-adrenoreceptors will differ in different regions of the body [6]. Consequently, it can be tentatively suggested on the basis of the observations described above that the density of α_1-adrenoreceptors in the precapillary sphincters is much greater than the density of α_2-adrenoreceptors, for effects of the selective α_1-agonist phenylephrine were considerably (2.2 times) stronger than the effect of the α_2-agonist clonidine.

Considering that α_1-adrenoreceptors, as was pointed out above, are concentrated mainly in the sphere of the neuroeffector synapse and react mainly with endogenous NA, the effect of stimulation of the superior cervical sympathetic nerve on the morphological and functional state of the cerebral cortical capillary system was studied in a series of experiments. The results showed that sympathetic nerve stimulation (SNS) increased the number of GCC by 147% compared with the control and reduced their diameter by 18.7%. The effects indicated above are much greater than those observed under the influence of clonidine and they confirm the conclusion that the density of α_1-receptors is greater than that of α_2-receptors in the precapillary sphincters of the cat cerebral cortex.

When the results of this study of relations between the adrenergic component and calcium antagonists are examined at the capillary system level, it must be pointed out that verapamil exhibits its action mainly when α_1-receptors are excited by phenylephrine. For instance, under the influence of verapamil the effect of phenyl-

ephrine on the precapillary sphincters was reduced by 47.1% and that of NA by 20%; however, it will be noted that the number of nonfunctioning capillaries still remained greater than in the control. Verapamil had hardly any influence on the effects of clonidine. It is an interesting fact that verapamil also reduced the influence on SNS on the number of nonfunctioning capillaries (Fig. 2), but in this case also abolition of the effect was partial in character (31.1%). The effects of nifedrine were stronger than those of verapamil. For instance, during simultaneous excitation of α_1- and α_2-receptors by NA, nifedrine reduced the number of nonfunctioning capillaries by 20% and increased their diameter a little. If α_1-receptors were selectively excited by phenylephrine, nifedrine reduced the number of nonfunctioning capillaries by 8.5 times compared with the effect of verapamil, and by 2.4 times compared with the control. The effect of excitation of α_2-receptors by clonidine was reduced by half by nifedrine. It must also be noted that nifedrine depressed the effect of SNS on the number of nonfunctioning capillaries by 2.3 times (Fig. 1).

When the results are assessed the following point must be taken into account: For contraction of the smooth muscle of blood vessels a definite threshold concentration of intracellular calcium is necessary. Since the intracellular calcium reserves are comparatively small in smooth muscles, the threshold calcium level is maintained chiefly by extracellular Ca^{++} through channels in the cell membranes [3]. When α_1- and α_2-adrenoreceptors are excited, passive Ca^{++} transport is intensified. Excitation of α_2-receptors opens the receptor-controlled channels, which are voltage-dependent, whereas the α_1-receptor opens voltage-independent Ca-channels [6]. Under the influence of NA both channels function, whereas phenylephrine acts chiefly through voltage-independent, and clonidine through dependent channels. Hence it follows that during activation of α-adrenoreceptors of precapillary sphincters, especially of α_1-receptors, the "external gates" of the Ca-channels play an important role, for nifedrine, which blocks the "external gates" gives rise to a marked degree of inhibition of the effect of both phenylephrine and SNS. The ability of nifedrine to inhibit effects of clonidine also is evidence of the important role of the "external gates" of Ca^{++} in functioning of the α_2-receptors of the precapillary sphincters, whose density, as was pointed out above, is evidently less than the density of α_1-receptors. Participation of the "internal gates" of Ca^{++} in the mechanism of the adrenergic response of the precapillary sphincters also will be evident, although less clearly manifested.

The Ca-channels thus play an important role in function of the precapillary sphincters, whose activity is largely dependent on activity of α-adrenoreceptors, in particular, of α_1-receptors. A more important role in the changes in basal tone of the precapillary sphincters under the influence of calcium antagonists is evidently played by the "gating mechanism" of the Ca-channels, whereas voltage-control and receptor-operated Ca-channels play the leading role in the maintenance of adrenergic tone.

LITERATURE CITED

1. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
2. A. N. Chilingaryan, Zh. Éksp. Klin. Med., 17, No. 5, 19 (1977).
3. D. F. Bohr and R. C. Webb, in: Mechanisms of Vasodilatation, Basal (1978), pp. 37-47.
4. H. Glossman, D. R. Ferry, F. Lübbecke, et al., Trends Pharmacol. Sci., 3, 431 (1982).
5. M. Göthert, P. Navroth, and I. M. Pohl, in: Proceedings of the 4th International Catecholamine Symposium, Vol. 1, New York (1979), pp. 289-291.
6. S. Z. Langer and N. B. Shepperson, Trends Pharmacol. Sci., 3, 440 (1982).
7. P. M. Vanhoutte, J. Cardiovasc. Pharmacol., 4, 91 (1982).