PHYSIOLOGY

EFFECT OF CHOLINERGIC ACTIVATION ON RESISTIVE AND CAPACITIVE FUNCTIONS OF THE CEREBRAL VESSELS

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There is evidence in the literature that acetylcholine induces endothelium-dependent dilatation of cerebral arteries [10, 13, 14], and that intravascular injection of acetylcholine leads to an increase in the total and local cerebral flow [10, 12], as a result of a decrease in the resistance of all subsequent parts of the cerebrovascular system [3, 10, 12] and an increase in permeability of the cerebral vessels [3, 12]. The arteries and veins of the brain possess a cholinergic innervation [11, 14], the anatomical sources of which have not yet been finally identified [6, 8], so that they cannot be acted upon selectively under experimental conditions. The use of intravital microscopy and luminescence histochemistry has shown that general activation of the cholinergic systems of the brain causes dilatation of the pial vessels [5] and also increases activity of the central sources [1, 15, 16] and intravascular mechanisms [7] of the adrenergic innervation of the cerebral arteries. However, this evidence is not sufficient to allow estimation of the real contribution of cholinergic influences to regulation of the cerebral circulation.

The aim of this investigation was to study changes in resistance and capacity of the cerebral vessels during generalized pharmacologic activation of endogenous cholinergic mechanisms and also the sensitivity of these vessels to catecholamines under the conditions described above.

EXPERIMENTAL METHOD

Altogether 15 acute experiments were carried out on cats anesthetized with urethane (1 μg/kg) during perfusion of the hemodynamically isolated brain by means of a constant delivery pump [2]. The extracranial arteries and veins were excluded from the circulation by dissection. The blood and CSF systems of the brain and spinal cord were isolated from one another by measured occlusion of the latter by compression, together with adjacent vessels, at the level of the atlanto-occipital joint. Heparinized (1500 U/kg) blood was pumped into the common carotid arteries and escaped via the external jugular veins into an extracorporeal reservoir, followed by oxygenation in the lungs of a donor cat. The resistive and capacitive functions of the cerebral vessels were investigated by resistography and accumulography [2, 3]. Changes in the total cerebrovascular resistance were judged from the level of the perfusion pressure, and changes in venous outflow from the blood level in the extracorporeal reservoir; the systemic arterial pressure of the donor cat, and in five experiments the CSF pressure in the subdural space of the perfused brain, also were recorded. A two-channel perfusion pump and the mechanotron electromanometers used in the work were made at the Experimental Production Workshop of the Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR.

The cholinergic systems of the brain were activated by injection of the organophosphorus cholinesterase inhibitor phosphacol [O,O-diethyl-O-(p-nitrophenyl)phosphate] (a 0.5% solution in a mixture of equal quantities of dimethyl sulfoxide and polyglucin) into the afferent channel of the pump. Cholinesterase activity was determined by measuring the rate of hydrolysis of acetylcholine in blood homogenate by an ETS-822 automatic potentiometer titration system (Radiometer, Denmark). In the course of the experiment phosphacol was injected in 3 or 4 portions, each of 150 μg/kg, with intervals

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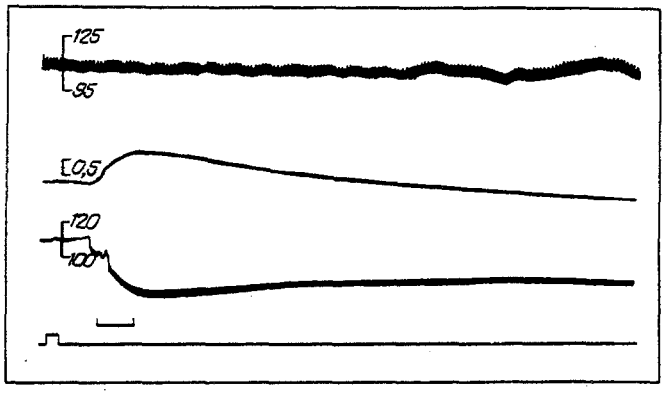

Fig. 1. Changes in perfusion pressure and venous outflow in response to injection of phosphacol (150 μg/kg) into cerebrovascular circulation. From top to bottom: systemic arterial pressure (donor cat), mm Hg; venous return from brain, ml/min; perfusion pressure, mm Hg; time marker – 20 sec; marker of injection.

TABLE 1. Changes in Parameters Reflecting Resistance, Capacity, and Permeability of Cerebral Vessels after Injection of Different Doses of Phosphacol (M ± m)

Parameter	Background	Dose of phosphacol injected, μg/kg			
		150	300	450	600
Perfusion pressure, T	100±5,7	−21,4±6,0	−16,1±4,0	−15,9±4,6	−13,9±6,0
Venous outflow, %	100±1,5	2,1±0,6	2,9±0,3	3,8±0,8	4,3±1,1
Increase in perfusion pressure in response to injection of noradrenalin, % of initial level					
Capillary pressure, %	19,0±2,4	13,6±2,4	15,4±2,3		32,4±4,5
Coefficient of capillary filtration, %	100±14,1		94,4±7,0		74,3±9,6
	100±13,3		90,9±7,8		82,5±8,4

of 30 min, ensuring gradual inhibition of activity of the blood cholinesterases by 15-20% relative to the previous injection. It was shown previously by histochemical methods and by cytospectrophotometry [7] that this parameter correlates with the acetylcholinesterase content in the nervous plexuses of the cerebral arteries.

The anticholinesterase action of phosphacol is irreversible in character and undergoes summation during repeated injections [4]. With this fact in mind, to assess the level of transcapillary migration of fluid in the perfused brain, the capillary hydrostatic pressure was determined between injections of phosphacol, and to estimate the area of the exchange surface and vascular permeability, the coefficient of capillary filtration was calculated [2, 9].

Reactivity of the cerebral vessels was judged from changes in perfusion pressure in response to injection of noradrenalin (5 μg in 0.1 ml physiological saline) into the afferent channel of the pump. Tests for reactivity to catecholamines were carried out after each injection of phosphacol.

The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

For 30 sec after injection of phosphacol the perfusion pressure was lowered on average by 17.[illegible] ± 2.6% compared with the initial level (121.6 ± 6.9 mm Hg, Fig. 1). The fall of pressure decreased with each subsequent injection, although the corresponding differences were not statistically significant (Table 1). Simultaneously with the reaction described above the venous outflow increased on average by 2.9 ± 0.3% of the volume velocity of perfusion, which was 24 ml/min in all the experiments; the increase in venous outflow, moreover, increased significantly from the first to the fourth injections (Table 1). For 10 min after injection of phosphacol the pressure and venous outflow returned to their initial values. No change in CSF or systemic arterial blood pressure of the donor cat was observed in response to the injection of phosphacol.

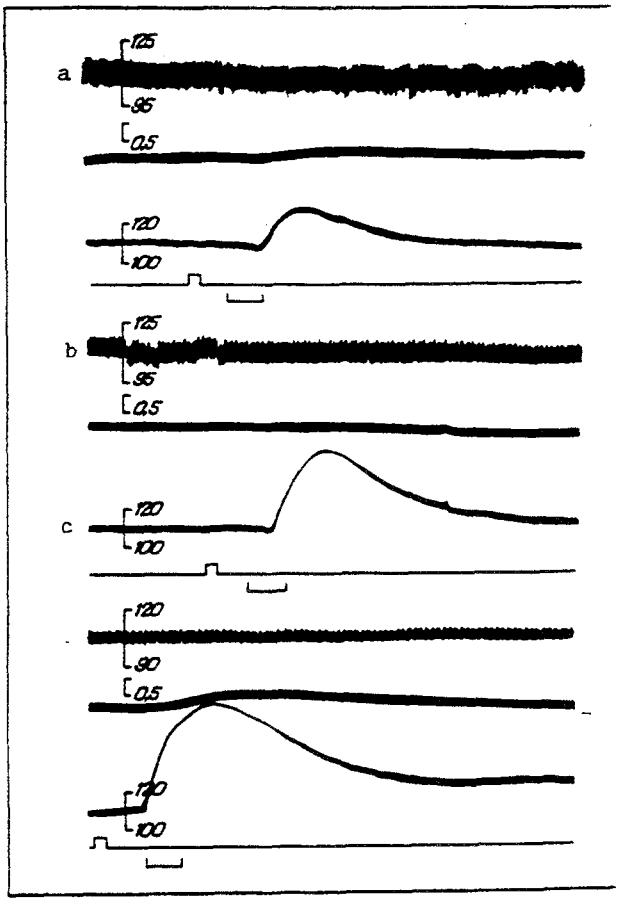

Fig. 2. Changes in perfusion pressure in response to injection of noradrenalin (5 μg in 0.1 ml of physiological saline) into cerebrovascular circulation. Legend as to Fig. 1: a) before injection of phosphacol, b and c) after injection of phosphacol in doses of 450 and 600 μ/kg respectively.

After one or two injections of phosphacol the capillary hydrostatic pressure in the cerebrovascular circulation showed no significant changes. After three or four injections it fell to 74.3 ± 9.6% of the initial value, which was 13.5 ± 1.9 mm Hg, at which level it subsequently remained. The coefficient of capillary filtration in the brain also showed a tendency to fall with an increase in the total dose of phosphacol injected, but this decrease relative to the initial level was statistically significant (Table 1).

The sensitivity of the cerebral arteries to noradrenalin depended on the total dose of phosphacol injected previously. From its initial value of 19.0 ± 2.4%, the rise of perfusion pressure induced by noradrenalin decreased somewhat after one or two injections of phosphacol, but after three or four injections it rose to almost twice the background value (Fig. 2; Table 1).

The investigations thus showed that general pharmacologic inhibition of cholinesterase activity reduces the resistance and capacity of the cerebral vessels, as is shown by reduction of the perfusion pressure and an accompanying increase in venous outflow. In the course of 10 min these parameters returned to normal despite the irreversible character of the anticholinesterase action of phosphacol. Changes similar to those described above, but greater in amplitude, in the perfusion pressure and venous outflow from the brain were previously observed under similar experimental conditions in response to injection of acetylcholine into the cerebrovascular circulation [3].

This state of affairs, and also the absence of information in the literature [4, 15] on the direct vasomotor action of organophosphorus cholinesterase inhibitors, suggests that the lowering of perfusion pressure observed in the experiments described above reflect neurogenic cholinergic dilatation of arterial vessels, whose existence in the brain has not hitherto been proved [8].

It is also known from the literature [3] that intravascular injection of acetylcholine causes lowering of the capillary hydrostatic pressure in the brain. According to our investigations, this type of effect is observed only after 3 or 4 injections of phosphacol, when cholinesterase activity does not exceed 10% of the normal value. Assuming the cholinergic neurogenic nature of the vascular reactions observed in this study, it seems probable that the short-term increase in venous outflow in response to injection of phosphacol depends on potentiation of the resistive function of the veins, whose cholinergic innervation and sensitivity to acetylcholine have so far received little study [2, 9]. Meanwhile the irreversible fall of the capillary hydrostatic pressure evidently reflects increased absorption of interstitial fluid, also caused by cholinergic influences of neurogenic origin.

The results of this investigation are evidence that inhibition of cholinesterase activity under the influence of high doses (450-600 μg/kg) of phosphacol leads to a twofold increase in sensitivity of the cerebral arteries to noradrenalin. This is evidence that cholinergic influences on the cerebral circulation may be realized and effected through a change in activity of the adrenergic systems at the level of forces of innervation [1, 16], of the intravascular nervous apparatus [7], and of effector cell membranes.

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