EFFECT OF METHYLAPOGALANTHAMINE ON TONE OF THE CEREBRAL, EXTRACRANIAL, AND PERIPHERAL VESSELS

V. E. Pogorelyi and M. D. Gaevyi

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Acute resistographic experiments on anesthetized and unanesthetized cats showed that methylapogalanthamine lowers the general arterial pressure and the tone of the cerebral, extracranial, and femoral vessels. The effect on the femoral vessels is the strongest. A biphasic (dilator-constrictor) response was frequently observed. Methylapogalanthamine weakened the constrictor response of the intra- and extracranial vessels to stimulation of the cervical sympathetic nerve. Anesthesia potentiated, whereas rausedil weakened the vasodilator effect of methylapogalanthamine.

KEY WORDS: circulation; cerebral vessels; rausedil.

According to the data in the literature [4, 5], methylapogalanthamine is an active hypotensive agent with adrenolytic and spasmolytic activity. Its ability to inhibit electrical activity of the mesencephalic reticular formation can be explained by its central adrenolytic action.

In the light of modern data on the role of the sympathico-adrenal system in the regulation of the cerebral circulation [1, 3, 6-8], the action of methylapogalanthamine on cerebral vascular tone is particularly interesting, but there is no information on this matter in the literature.

In the investigation described below the action of methylapogalanthamine on cerebral vascular tone was compared with its action on the extracranial and peripheral vessels.

EXPERIMENTAL METHOD

Acute experiments were carried out on 30 adult cats of both sexes under general urethane anesthesia (1 g/kg, intravenously) or under local procaine anesthesia supplemented by muscle relaxants (succinylcholine, diplacin) and artificial respiration. The tone of the cerebral vessels was recorded by means of a four-channel resistograph, which was connected to the vessels for study. Isolated perfusion of the cerebral vessels and of the extracranial vessels of the head was ensured by appropriate ligation of the carotid and vertebral systems [2]. Vessels of the hind limb were perfused by Khayutin's method [10]. The perfusion pressure and systemic arterial pressure (in the carotid artery) were recorded with mercury manometers. Heparin was used as anticoagulant. Methylapogalanthamine (0.03% solution) was injected intravenously (0.1 mg/kg) and intra-arterially (0.01 mg/kg into the perfusion system). In some experiments before and after intravenous injection of methylapogalanthamine the cervical sympathetic nerves were stimulated electrically with square pulses (30 Hz, 1.5 msec, 5 V) for 15 sec.

EXPERIMENTAL RESULTS

Intravenous injection of methylapogalanthamine (0.1 mg/kg) into anesthetized cats (eight experiments) caused an initial fall in the tone of the cerebral vessels by 18.9 ± 2.1% ($P < 0.001$), of the extracranial vessels by 23.8 ± 1.8% ($P < 0.001$) and of the vessels of the hind limb by 31.3 ± 4.7% ($P < 0.001$). The effect began immediately after injection of the drug, it was strongest during the first minute, and its mean duration was 10, 30, and 45 min, respectively in the vessels indicated above. Between 10 and 15 min after re-establishment of the initial level of perfusion pressure a second (constrictor) phase often began, and continued until the end of the experiments (duration of observations 60-90 min). The constrictor

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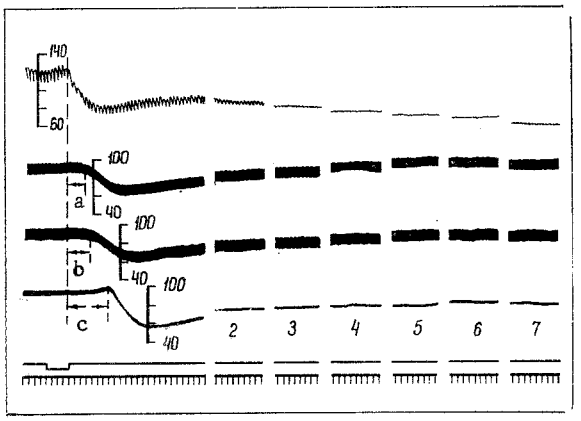

Fig. 1

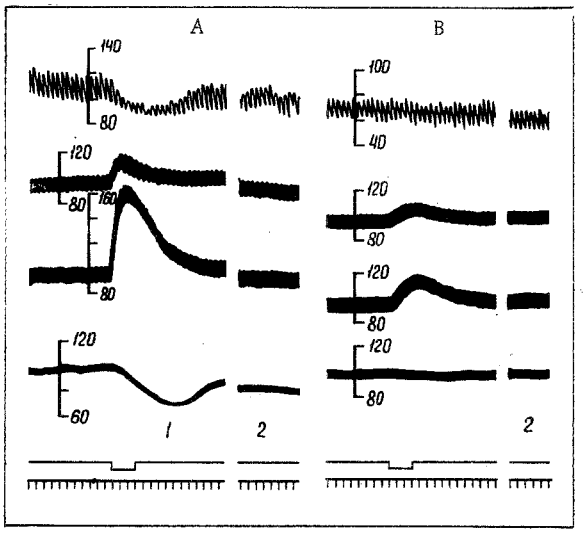

Fig. 2

Fig. 1. Effect of methylapogalanthamine (0.1 mg/kg, intravenously) on tone of cerebral, extracranial, and femoral vessels and on systemic arterial pressure. From top to bottom: systemic arterial pressure; resistogram of intracranial vessels; resistogram of extracranial vessels, resistogram of femoral vessels (a, b, c — results of deliberate increase in volume of perfused channels); marker of injection of methylapogalanthamine; time marker 5 sec. 1) At moment of injection; 2-7) 3, 5, 10, 30, 60, and 90 min later, respectively.

Fig. 2. Vascular responses to electrical stimulation of cervical sympathetic trunk before (A) and after (B) injection of methylapogalanthamine. 1) At moment of electrical stimulation, 2) 3 min later. Remainder of legend as in Fig. 1.

phase was most clearly manifested in the cerebral vessels. The systemic arterial pressure fell immediately after injection of methylapogalanthamine to 29.1 ± 3.1% of its initial level after 1 min, rising again to 48.2 ± 8.1% after 60 min ($P < 0.001$). The character of the vascular responses to intravenous injection of methylapogalanthamine is illustrated in Fig. 1.

In the experiments in which a method of demonstrating the direct and indirect action of pharmacological agents on the blood vessels [9] was used, "delay" (the intervals a, b, and c in Fig. 1) of the response of the perfused vessels to injection of methylapogalanthamine was observed, corresponding to the deliberate increase in volume of the perfusion channels. This points to the direct action of the drug on the blood vessels.

In six experiments the cervical sympathetic nerves were stimulated before (control) and after injection of methylapogalanthamine (Fig. 2). Control stimulation caused the perfusion pressure to rise in the cerebral vessels by 32.7 ± 12.5% ($P < 0.001$) and in the extracranial vessels by 47.7 ± 10.3% ($P < 0.001$). The perfusion pressure in the vessels of the hind limb and the systemic arterial pressure fell by 21.3 ± 5.4% ($P < 0.001$) and 14 ± 3.5% ($P < 0.001$) respectively. Repeated stimulation of these same nerves 60 min after injection of the drug caused the perfusion pressure in the cerebral and extracranial vessels to rise by 10.5 ± 4.8% ($P = 0.05$) and 12 ± 5.4% ($P = 0.05$) respectively. Practically no effect was observed on the femoral vessels and systemic arterial pressure. Consequently, methylapogalanthamine considerably reduces the sympathetic vasoconstrictor responses and completely abolishes depressor responses to stimulation of sensory fibers of sympathetic nerves.

Intra-arterial injection of methylapogalanthamine (0.01 mg/kg) into anesthetized cats (seven experiments) caused an immediate decrease in perfusion pressure in the cerebral, extracranial, and femoral vessels by 25.4 ± 4.2% ($P < 0.001$), 23.0 ± 3.5% ($P < 0.001$), and 30.0 ± 5.1% ($P < 0.001$) respectively. The action of the drug was strongest during the first minute, and the duration of its action varied within fairly wide limits (from 3 to 20 min). As a rule the perfusion pressure in the cerebral vessels returned to its initial level much sooner than that in the extracranial and femoral vessels. In some experiments a biphasic (dilator-constrictor) response was observed. The systemic arterial pressure 1-2 min after injection of the drug began to fall gradually, and by the tenth minute the mean hypotensive effect was 29%. In some experiments the hypotensive effect was preceded by a transient (30-90 sec) and moderate hypertensive response.

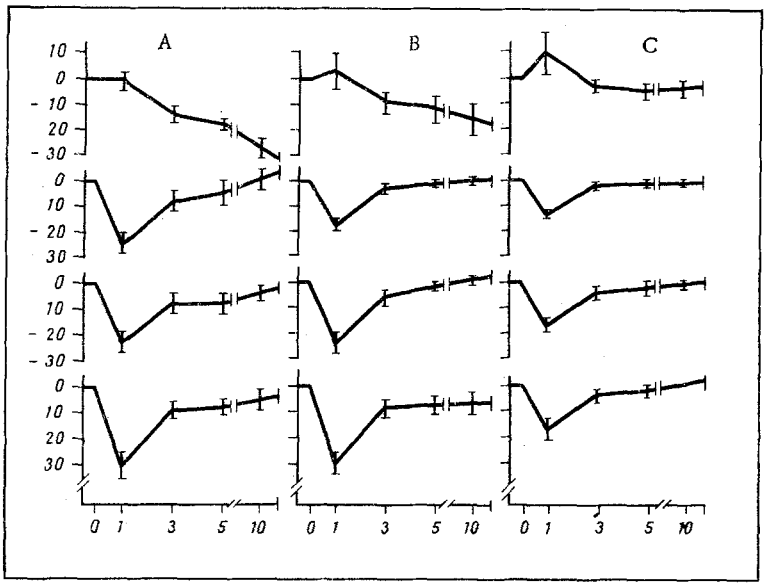

Fig. 3. Vascular responses to injection of methylapogalanthamine (0.01mg/kg, intra-arterially) in anesthetized (A) and unanesthetized (B) animals and after administration of reserpine (C). Abscissa, time of testing (in min); ordinate, changes (in % of initial level). From top to bottom: systemic arterial pressure; resistogram of intracranial vessels; resistogram of extracranial vessels; resistogram of femoral vessels.

To study how the vascular effects of methylapogalanthamine depend on the functional state of the CNS vascular responses were compared in anesthetized and unanesthetized (local anesthesia, muscle relaxants) animals and after premedication with reserpine (Fig. 3).

The results of these experiments showed that intra-arterial injection of the same dose of methylapogalanthamine (0.01 mg/kg) into unanesthetized animals (eight experiments) gave analogous vascular effects, but the response of the cerebral vessels and of the systemic arterial pressure was less marked than in anesthetized animals. Marked weakening of the vascular responses to injection of methylapogalanthamine was observed in the unanesthetized animals which had received a preliminary (2-4 h before injection of methylapogalanthamine) intravenous injection of the sympatholytic rausedil in a dose of 1-2 mg/kg.

It can be concluded from these results that methylapogalanthamine causes a biphasic (dilator-constrictor) response of the cerebral vessels. Weakening of the constrictor effect on the intra- and extracranial vessels in response to stimulation of the sympathetic nerves by methylapogalanthamine is evidence of the antiadrenergic action of the drug. Potentiation of the dilator vascular effects of methylapogalanthamine on anesthetized animals and their weakening after preliminary injection of rausedil can be explained by the participation of central and peripheral mechanisms in the realization of the vascular effects of methylapogalanthamine.

LITERATURE CITED

1. E. S. Gabrielyan, Some Aspects of the Physiology and Pharmacology of the Cerebral Circulation [in Russian], Erevan (1976).
2. M. D. Gaevyi and S. P. Trofimenko, Fiziol. Zh. SSSR, No. 10, 1457 (1970).
3. M. D. Gaevyi, Fiziol. Zh. SSSR, No. 11, 1677 (1970).
4. U. B. Zakirov, Kh. U. Aliev, and I. K. Kalimov, Farmakol. Toksikol., No. 6, 708 (1972).
5. M. D. Mashkovskii, Therapeutic Substances [in Russian], Vol. 1, Moscow (1977), pp. 406-407.
6. R. S. Mirzoyan, "The pharmacology of adrenergic regulation of the cerebral circulation," Author's Abstract of Doctoral Dissertation, Moscow (1977).
7. G. I. Mchedlishvili, Functions of the Vascular Mechanisms of the Brain. Their Role in the Regulation and Pathology of the Cerebral Circulation [in Russian], Leningrad (1968).

8. G. I. Mchedlishvili, Spasm of the Arteries of the Brain [in Russian], Tbilisi (1977).
9. V. M. Khayutin, Farmakol. Toksikol., No. 3, 78 (1958).
10. V. M. Khayutin, Vasomotor Reflexes [in Russian], Moscow (1964), p. 93.

EFFECT OF REVERSIBLE INHIBITORS ON REACTIVITY OF FUNCTIONAL SITES OF THE ACTIVE CENTER OF ACETYLCHOLINESTERASE

V. D. Tonkopii and T. V. Ardab'eva

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The effect of galanthamine, tacrine, and oxazyl* on phosphorylation of acetylcholinesterase of human erythrocytes by armin† and GD-42 (o-ethyl-S-β-ethylthioethyl ester of methylthiophosphinic acid) was studied. In the presence of galanthamine the organophosphorus inhibitors were shown to interact only with the binding site of the enzyme whose anionic region was not occupied by the reversible inhibitor. Tacrine and oxazyl lower the reactivity of the free enzyme and reduce the rate of its phosphorylation.

It is now accepted that there are two functional sites — anionic and esterase — on the active surface of acetylcholinesterase (AChE). In the presence of tetraalkylammonium ions, competitive reversible inhibitors of AChE, the molecule of an organophosphorus inhibitor (OPI) does not interact with a binding site whose anionic region is occupied by the reversible inhibitor [4]. In recent years abundant experimental data have been obtained to show the existence of noncatalytic allosteric sites and hydrophobic zones on the surface of AChE [2, 11, 12]. The effect of reversible inhibitors interacting with AChE in the noncatalytic regions on the reactivity of the binding site of the enzyme has not yet been adequately studied [10].

In the investigation described below the effect of reversible AChE inhibitors — galanthamine, tacrine, and oxazyl* — on subsequent phosphorylation of the esterase region by armin† and GD-42 (o-ethyl-S-β-ethylthioethyl ester of methylthiophosphinic acid) was investigated.

EXPERIMENTAL METHOD

The source of enzyme was a purified preparation of AChE from human erythrocytes obtained from the Perm' Institute of Vaccines and Sera, with an activity of 2.1 units/mg. Galanthamine hydrobromide, tacrine (9-amino-1,2,3,4-tetrahydroacridine), and oxazyl were used as reversible inhibitors. Inhibition constants (K_i) for AChE by these compounds were determined by the method of Lineweaver and Burk [7]. The velocity of enzymic hydrolysis of acetylcholine (ACh) was determined by continuous potentiometric titration with two burets [3] on the TTT-1c automatic titrator (Radiometer, Denmark) in the presence of 0.07 N KCl solution at pH 7.5 and 37°C.

The velocity of irreversible inhibition of activity of the enzyme by OPI was assessed under the same experimental conditions from the values of bimolecular reaction velocity constants in the presence of reversible inhibitors ($K_{II,i}$) and in their absence (K_{II}) and calculated by the equation for a pseudomonomolecular reaction:

$$K_{II} = \frac{2.3}{[I]} \cdot \lg \frac{v_0}{v_t},$$

*Bis-(o-chlorobenzyldiethylammoniumethyl)-oxamide dichloride.
†Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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