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ELECTROPHYSIOLOGICAL ANALYSIS OF THE ACTION OF CAVINTON ON SMOOTH MUSCLES

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Cavinton, the ethyl ester of apovincamic acid, is a synthetic derivative of alkaloids of the lesser periwinkle *Vinca minor* and it is used in chemical practice for the treatment of diseases due to cerebrovascular disorders.

The therapeutic action of cavinton is associated with its ability to dilate mainly cerebral vessels. Although there have been many investigations on the clinical aspects of the use of cavinton, the mechanism of its action on smooth muscles has hardly been studied at all [3-7].

The aim of this investigation was a comparative analysis of the action of cavinton on smooth muscles of various organs, using electrophysiological techniques of investigation, whereby changes in membrane potential and membrane conductance of various ions could be monitored, with simultaneous recording of the contractile responses of muscle strips.

EXPERIMENTAL METHOD

The electrophysiological investigations were conducted on spiral muscular strips of bovine cerebral (basilar and posterior communicating) arteries, longitudinal strips from the rabbit portal vein, and the guinea pig taenia coli, by the sucrose gap method, with simultaneous recording of contractions of the muscle strips by means of a mechanotron [1]. The length of the muscle strips was not more than 10 mm and their width 1 mm. The composition of the Krebs' solution was as follows (in mmoles/liter): NaCl, 120.4; KCl, 5.9; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2, $CaCl_2$, 2.5; glucose 11.5. The cavinton used in the experiments was obtained from Gedeon Richter (Hungary). In the experiments with the hyperpotassium solution, the potassium ion concentration was increased to 80 mmole/liter by the addition of the dry HCl salt to the Krebs' solution. The temperature of the surrounding solution during the tests was 36°C and its pH was 7.4. Electrical and contractile activity of the smooth muscles

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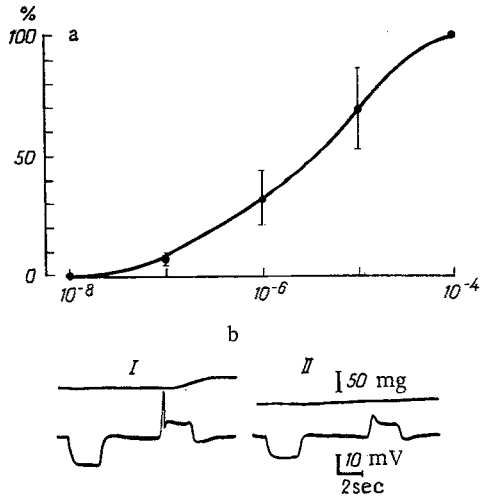

Fig. 1

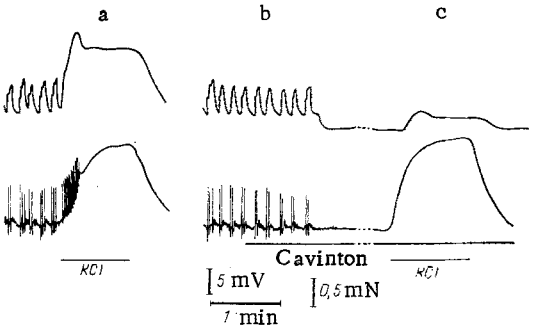

Fig. 2

Fig. 1. Action of cavinton on muscle strips from cerebral arteries: a) dependence of relaxation of muscle strips on cavinton concentration in Krebs' solution. Maximal relaxation observed with cavinton in a concentration of 10^{-4} mole/liter taken as 100%; b) fragments of trace of contractile activity and an- and catelectrontonic potentials in normal Krebs' solution (I) and in the presence of cavinton, 10^{-4} mole/liter (II), recorded from oscilloscope screen. Electrotonic potentials in both cases evoked by electric pulses of equal strength. Here and in Figs. 2 and 3, top trace shows changes in contractile activity, bottom trace — transmembrane potential.

Fig. 2. Action of cavinton (10^{-4} mole/liter) on electrical and contractile activity of portal vein muscle cells: a) action of hyperpotassium solution (80 mmoles/liter) on spontaneous activity; b) action of cavinton; c) action of hyperpotassium Krebs' solution in the presence of cavinton.

was recorded simultaneously on KSP-4 graph paper and simultaneously on photographic film from the screen of an S1-16 oscilloscope by means of an FOR-2 camera.

EXPERIMENTAL RESULTS

Of the muscle preparations investigated, smooth muscles of the cerebral arteries were most sensitive to the action of cavinton. The muscle cells of these blood vessels in normal Krebs' solution did not possess spontaneous activity, but in response to stimulation by a depolarizing current they could generate single action potentials (AP), accompanied by phasic contractions. During administration of cavinton (10^{-7}-10^{-4} mole/liter) dose-dependence relaxation of the muscle strips took place (Fig. 1a) without any appreciable change in resting potential (RP). With cavinton in a concentration of 10^{-4} mole/liter relaxation of the muscle strips was maximal and near to the relaxation induced by adenosine in the same concentration. The action of cavinton in concentrations over 10^{-5} mole/liter was accompanied by a decrease in the resistance of the muscle cell membranes and by appreciable inhibition of AP evoked by electrical depolarization of the membrane (Fig. 1b). Against the background of relaxation induced by cavinton in concentrations of not more than 10^{-5} mole/liter, electrical and contractile responses of the muscle strips were unchanged by the action of the hyperpotassium solution. However, when cavinton was used in higher concentrations the AP arising at the beginning of potassium depolarization and the phasic contractions were inhibited, and the tonic component of the potassium contraction was considerably reduced.

By contrast with the smooth muscle cells (SMC) of the cerebral arteries, SMC of the portal vein and taenia coli were insensitive to the action of cavinton in concentrations below 10^{-5} mole/liter. Meanwhile these muscles, like the cerebral arteries, responded to higher concentrations of cavinton by a decrease in amplitude of the AP evoked by the depolarizing current and in the resistance of the membrane, without any appreciable changes in RP. In the initial state of portal vein muscle cells exhibited spontaneous activity. Phasic contractions accompanying each AP underwent summation into incomplete tetanus and the muscle cells

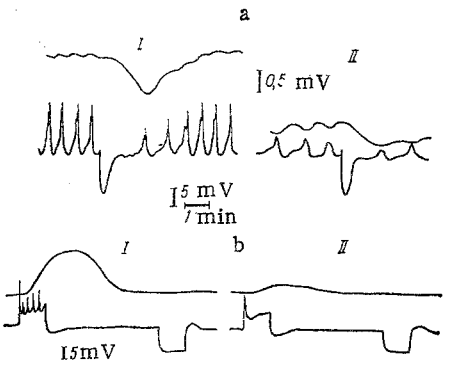

Fig. 3. Action of cavinton on muscle cells of guinea pig taenia coli: a) spontaneous electrical and contractile activity, and inhibitory synaptic potentials of muscle cells in normal Krebs' solution (I) and against the background of cavinton in a concentration of 10^{-4} mole/liter (II). Time of stimulation of intramural nerve fibers indicated by a dot; b) electrotonic potentials in normal Krebs' solution (I) and under the influence of cavinton (II). Electrotonic potentials in both cases induced by electric pulses of equal strength. Photographed from oscilloscope screen.

were in a state of tetanic contraction. Cavinton (10^{-4} mole/liter) inhibited spontaneous AP and phasic contractions, and this was accompanied by lowering of the tetanic tone of the muscle strips (Fig. 2b). If the K^+ ion concentration in Krebs' solution was increased to 80 mmole/liter, this led to depolarization of the membrane of the protal vein SMC, with an increase in the frequency of AP at its beginning, accompanied by summation of phasic contraction of the muscle strip. These changes in electrical and contractile activity of SMC were then replaced by stable depolarization and prolonged tonic contraction of the muscle strip (Fig. 2a). In the presence of cavinton a hyperpotassium solution caused stable depolarization of SMC without the appearance of AP at its beginning, accompanied by tonic contraction of the muscle, the intensity of which was much less than in the control (Fig. 2c).

SMC of the guinea pig taenia coli also possessed spontaneous activity. Like SMC of the portal vein, they were initially in a state of tetanic contraction, maintained by frequent spontaneous AP. Cavinton (10^{-4} mole/liter) considerably reduced the amplitude and frequency of the spontaneous AP, as a result of which the tetanic tone of the muscle strip was reduced (Fig. 3a). In some cases spontaneous activity was completely inhibited by cavinton, but SMC of the taenia coli, like portal vein SMC, remained capable of generating modified single AP, accompanied by phasic contractions, in response to electrical stimulation (Fig. 3b).

The action of cavinton on neuromuscular transmission in SMC of the taenia coil also was investigated. Inhibitory synaptic potentials were evoked by stimulation of intramural nerve fibers by single pulses 0.2 msec in duration. Cavinton (10^{-4} mole/liter) reduced only the amplitude and frequency of the spontaneous AP, without affecting the inhibitory synaptic potentials (Fig. 3a).

In the modern view, stable relaxation of smooth muscles takes place either by a fall in the intracellular Ca^{++} ion concentration, on which the degree of activation of the contractile mechanism depends, or by inhibition of the activation of contraction itself [9, 11]. Ca^{++} ions can enter cells along three types of Ca channels: voltage-dependent inactivated, voltage-dependent uninactivated, and chemically controlled [8]. It is thus evident that identification of the point of application of cavinton, leading to relaxation of smooth muscles, is a difficult task. The relaxing action of cavinton might be linked with its recently discovered ability to inhibit activity of phosphodiesterase, isolated from various organs including smooth muscles of cerebral vessels [5]. Inhibition of phosphodiesterase activity causes an increase in the intracellular cAMP concentration, and it may thus be the cause of subsequent cAMP-dependent relaxation of SMC [9, 10]. However, relaxation of the cerebral vessels which we observed took place in the presence of low concentrations of cavinton (10^{-7}-10^{-5} mole/liter), at which inhibition of phosphodiesterase activity is not yet significant [5]. In other types of smooth muscles, for which high doses of cavinton (10^{-4} mole/liter) were more effective, its action likewise cannot be linked with inhibition of phosphodiesterase, for relaxation of muscle strips observed in these cases was connected with reduction of tetanic tone due to a decrease in the frequency and amplitude of AP. Reduction of excitability of SMC of all the types of smooth muscles studied, as well as the marked inhibition of contractile responses to hyperpotassium depolarization can evidently be attributed to the nonspecific action of high concentrations of cavinton on the voltage-dependent inactivated and uninactivated Ca channels of SMC. The absence of any effect of cavinton, in all concentrations, on inhibitory synaptic potentials is evidence that its action cannot be effected indirectly through neuromuscular transmission.

The writers showed previously [2] that the initial basal tone in SMC of the cerebral arteries has two components. Most of the tone (over 80%) is created by the inflow of Ca^{++} ions through chemically controlled Ca channels, which are open in the initial state. The reaminder of the tone is activated by Ca^{++} ions entering the muscle cells through voltage-dependent uninactivated Ca channels, which also are open at the resting potential of SMC. Conduction of the chemically sensitive Ca channels is controlled by adenosine-sensitive chemoreceptors. Activation of the chemoreceptors by adenosine leads to closing of these channels. As a result the inflow of Ca^{++} ions into the muscle cells is reduced and the basal tone is lowered. The present investigations showed that cavinton, in concentrations not exceeding 10^{-5} mole/liter, causes relaxation of SMC of the cerebral vessels similar in magnitude to the relaxation induced by adenosine in the same concentration. Since cavinton in these concentrations does not block the voltage-dependent inflow of Ca^{++} ions into SMC of the cerebral arteries, and since its action through intracellular mechanisms is unlikely, it can be tentatively suggested that its effect is connected with blocking of the chemosensitive, i.e., the adenosine-sensitive entry of Ca^{++} ions into SMC. This is possible if every initially open chemosensitive Ca channel is controlled by two different chemoreceptors. The alternative possibility is that adenosine and cavinton are agonists for the same chemoreceptors. Our results are not yet sufficient to eliminate one of these possibilities. Nevertheless, it is an essential fact that, of all types of smooth muscles investigated, relaxation which can be linked with blocking of the chemosensitive inflow of Ca^{++} ions into the muscle cells by cavinton, was obtained only in SMC of the cerebral vessels. This evidently also explains the selective action of cavinton on the cerebral blood flow. From the point of view of the clinical use of cavinton, a no less important fact is the discovery that its selective action on the cerebral vessels is manifested only in low concentrations, not exceeding 10^{-5} mole/liter. In higher concentrations caviton has a nonspecific action, manifested as partial inhibition of excitability in all the types of smooth muscles investigated. However, the end result of this action depends on the specific properties of the SMC of the different organs.

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