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EFFECT OF ATP AND ADENOSINE ON SPONTANEOUS ELECTRICAL AND CONTRACTILE ACTIVITY OF PORTAL VEIN SMOOTH MUSCLE CELLS

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Blood vessels are highly sensitive to adenine nucleotides, although their mechanism of action has not yet been explained. Interest in the vasomotor effects of adenine-containing substances has increased in the last decade, in particular, following the discovery of purinergic neuromuscular transmission in smooth muscles. According to recent investigations, the mediator for this transmission is evidently ATP [2], the inhibitory effect of which on smooth muscle cells of the gastrointestinal tract is effectively blocked by apamin [1]. The existence of purinergic neuromuscular transmission has also been postulated in smooth muscles of blood vessels [3].

The object of this investigation was to study the action of ATP and adenosine (AD) on spontaneous contractile and electrical activity of the smooth-muscle cells of the portal vein and also the action of apamin on the vascular effects of ATP and AD.

EXPERIMENTAL METHOD

Experiments were carried out on smooth-muscle cells of the portal vein of guinea pigs, rats, and rabbits. Longitudinal strips not more than 1.5 mm wide and 7 mm long were cut from the blood vessel. The muscle strip was kept under isometric conditions of contraction. To record electrical potentials the single sucrose gap method was used. Contractile activity was recorded by the 6-MKhlS mechanotron. Electrical potentials and contractile activity were recorded on a type KSP-4 automatic potentiometer.

Continuously flowing Krebs' solution (36°C, pH 7.4) used in the experiments had the following composition: NaCl 120.4 mM, KCl 5.9 mM, $NaHCO_3$ 5.5 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 2.5 mM, glucose 11.5 mM (made up in bidistilled water). The action of AD and ATP in concentrations of 10^{-6}-10^{-3} M was studied on spontaneously active muscle strips from the portal vein. Apamin, a polypeptide from bee venom, was used in a concentration of 10^{-7}-10^{-6} M. Because of the development of desensitization of the portal vein muscle cells to AD and ATP, an interval of 15-20 min was provided between the repeated action of these substances.

EXPERIMENTAL RESULTS

Under the influence of AD (10^{-6}-10^{-5} M) a small increase in the frequency of a spontaneous action potentials (AP) and of phasic contractions of the muscle strip of the guinea

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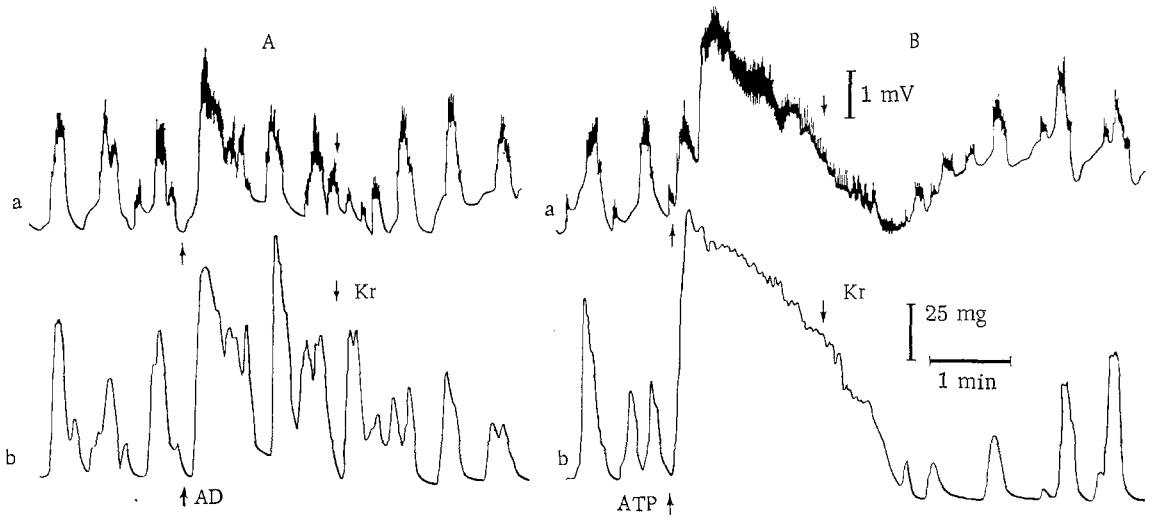

Fig. 1. Effect of AD (A) and ATP (B) in a concentration of 10^{-4} M on spontaneous electrical and contractile activity of smooth muscle cells of guinea pig portal vein. Here and in Figs. 2 and 3: a) spontaneous electrical activity; b) contractile activity of portal vein muscle cells. Arrows indicate beginning and end of action of substance.

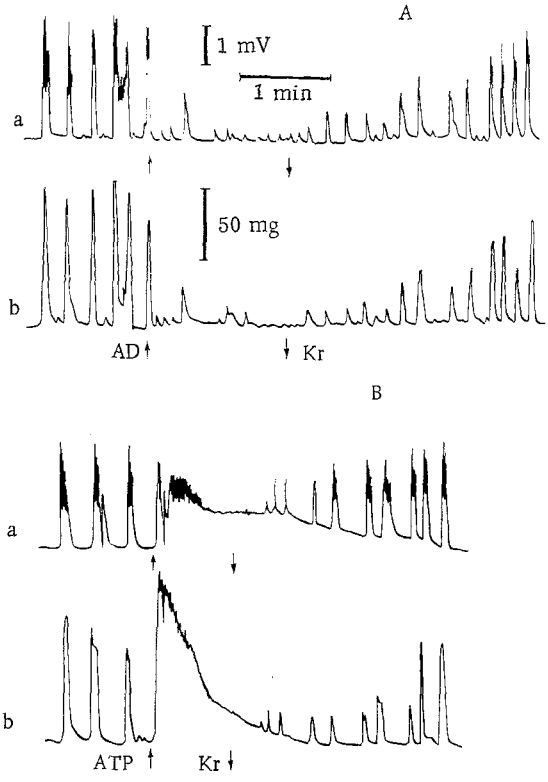

Fig. 2. Effect of AD (A) and ATP (B) on spontaneous electrical and contractile activity of smooth muscle cells of rat portal vein.

pig portal vein was observed. An increase in the AD concentration to 10^{-4}-10^{-3} M led to temporary depolarization of the membrane to 1.5 mV or more, and to a marked increase in the frequency of AP and of phasic contractions; the amplitude of the contractions, however, increased (Fig. 1A).

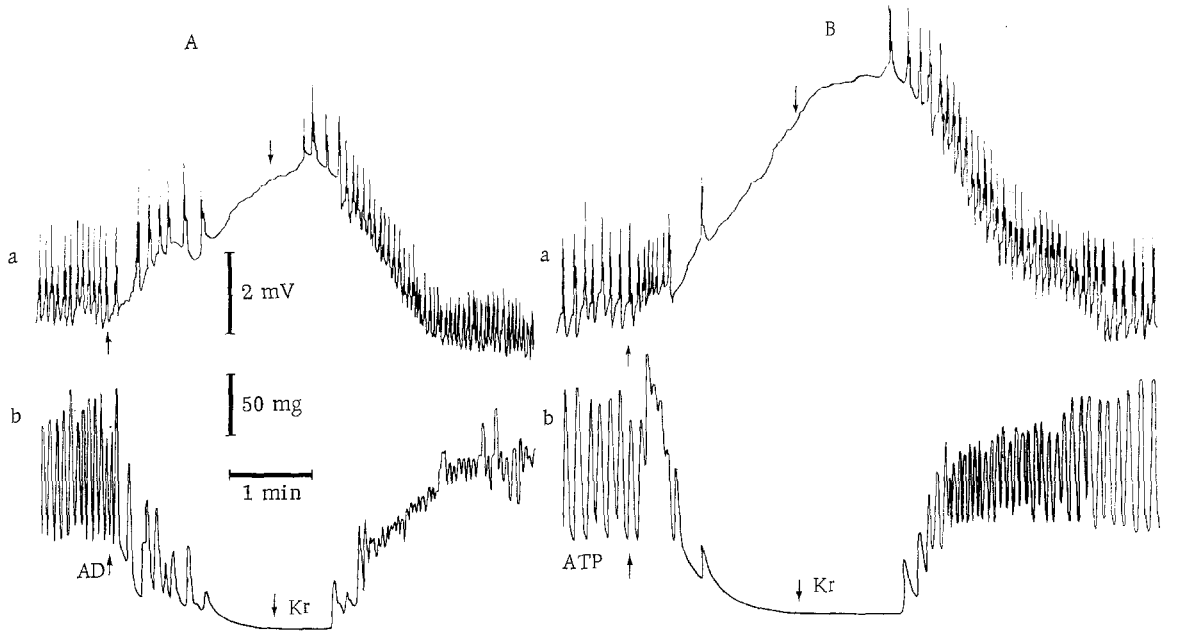

Fig. 3. Effect of AD (A) and ATP (B) on spontaneous electrical and contractile activity of smooth muscle cells of rabbit portal vein.

ATP (10^{-6}-10^{-5} M) also increased the frequency of AP and phasic contractions. An increase in ATP concentration to 10^{-4}-10^{-3} M led to temporary membrane depolarization to 4 mV, to an increase in the frequency of AP, and to tonic contraction of the muscle strip (Fig. 1B).

AD (10^{-6}-10^{-3} M) caused no visible changes in the resting potential (RP) of the muscle cells of the rat portal vein. Under the influence of AD (10^{-6}-10^{-5} M) an increase in the frequency of AP and of phasic contractions was observed, but with some decrease in their amplitude. An increase in the AD concentration to 10^{-4}-10^{-3} M led to considerable inhibition of AP and of phasic contractions of the muscle strip (Fig. 2A).

ATP (10^{-6}-10^{-5} M) had no effect on RP of the muscle cells but led to some increase in the frequency of AP and phasic contractions. An increase in the ATP concentration to 10^{-4}-10^{-3} M was accompanied by membrane depolarization to 2 mV and an increase in the frequency of AP and phasic contractions, followed by their inhibition (Fig. 2B).

Neither AD nor ATP, in concentrations of 10^{-6}-10^{-5} M, caused any visible changes in RP of the muscle cells of the rabbit portal vein. A decrease in the frequency of spontaneous AP and phasic contractions and also relaxation of the muscle strip were observed. AD (10^{-4}-10^{-3} M) evoked depolarization of the muscle cell membrane to 6 mV and a decrease in the frequency of AP or to complete suppression of their generation. These changes in electrical activity were accompanied by disappearance of phasic contractions and relaxation of the muscle strip (Fig. 3A). Under the influence of ATP (10^{-4}-10^{-3} M) a biphasic effect was observed: An increase in the frequency of AP and of phasic contractions was followed by inhibition of AP and relaxation of the muscle strip (Fig. 3B).

Changes in electrical and contractile activity of portal vein muscle cells in the experimental animals due to the action of AD and ATP were reversible. Spontaneous activity of the smooth muscle was completely restored by rinsing the preparations with Krebs' solution.

Apamin (10^{-7}-10^{-6} M) had no effect on spontaneous activity of the portal vein muscle cells of the experimental animals and did not change their response to ATP and AD.

The experiments showed that AD and ATP have an excitatory or inhibitory effect on muscle cells of the portal vein depending on the concentration of the compound and the species of the animal.

Both ATP and AD (10^{-6}-10^{-3} M) had a purely excitatory action on muscle cells of the guinea pig portal vein, as shown by temporary depolarization of the membrane, an increase in the frequency of AP and phasic contractions, and the onset of tonic contraction (Fig. 1). The observed depolarization is probably linked with an increase in permeability of the cell membrane to Na or Cl ions or to both simultaneously. ATP had a similar excitatory action on

the portal vein of the rat and rabbit. An increase in the frequency of spontaneous phasic contractions due to the action of ATP on the rat portal vein has also been observed by other workers [5]. As a result of the excitatory effect of ATP and AD, it must be noted, some degree of desensitization took place, as shown by the transient character of changes in RP and the decrease in depolarization observed during the repeated action of the test substances.

The inhibitory action of ATP and AD was most marked on the rabbit portal vein. Relaxation of a muscle strip from the portal vein under the influence of AD and ATP also was described by Su [8, 9]. This inhibition of electrical activity is most likely to be the cause of relaxation of the muscle strip, the tone of which is maintained in normal Krebs' solution by summation of fast phasic contractions. Since phasic contractions in the rat portal vein do not undergo summation because their frequency is too low, inhibition of AP under the influence of AD leads only to disappearance of the phasic contractions without causing relaxation of the muscle strip. The same phenomenon also was observed during the action of AD and ATP on the rabbit portal vein if the frequency of the original spontaneous activity of the muscle cells was low. It must also be noted that inhibition of electrical activity of the muscle cells under the influence of AD and ATP was not connected with inactivation of excitation as a result of their depolarization, for rinsing the strip led to restoration of AP, despite the fact that depolarization continued at the same level (Fig. 3). Moreover, under the influence of AD on the rat portal vein inhibition of AP generation was observed without any appreciable changes in RP of the muscle cells (Fig. 2A). It follows from these results that the inhibitory action of AD and ATP was evidently connected with their influence on the mechanism of AP generation.

Differences in reactions of the muscle cells to AD and ATP depending on their concentration and on the species of the animal, and also the distinct desensitization to their excitatory action, may indicate that the action of these substances is mediated through specific chemoreceptors in the muscle cell membrane. Since the inhibitory effect of ATP and AD, unlike their excitatory effect, does not exhibit desensitization it can be tentatively suggested that these effects are mediated by different chemoreceptors. The existence of purine receptors in the muscle cell membrane of blood vessels has been postulated by other workers [3, 4]. Evidence in support of this hypothesis is given by data showing that the action of AD is limited to the surface membrane of smooth-muscle cells [4].

The experimental data suggest that inhibitory chemoreceptors in the muscle cell membrane of the portal vein are connected with potential-dependent ion channels responsible for AP generation, whereas excitatory chemoreceptors are connected with potential-independent chemosensitive ion channels. A connection between the chemoreceptor and potential-dependent channels has also been found during investigation of the action of noradrenalin and histamine on muscle cells of the ureter [6, 7]. If both types of chemoreceptors are present, the character of the response will depend on their numerical proportions and on differences in the sensitivity of these receptors to AD and ATP.

The absence of a blocking action of apamin on the excitatory and inhibitory effects of ATP and AD in the portal vein may indicate that the purine receptor — ion channel complexes differ in nature in smooth muscles of the portal vein and of the gastrointestinal tract.

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