Papaverine enhances the negative inotropic effect of acetylcholine in rat auricles[1]

H. Nawrath

Department of Pharmacology, University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz (Federal Republic of Germany), 19 October 1977

Summary. The negative inotropic effect of acetylcholine in rat left auricles is enhanced in the presence of the phosphodiesterase inhibitor papaverine. This result favours the idea of a cyclic GMP-mediated action of acetylcholine in the heart.

Cyclic GMP may help mediate the effects of acetylcholine in atrial heart muscle. This assumption is supported by the observations that cyclic GMP levels are increased in response to acetylcholine[2,3] and that 8-bromo-cyclic GMP mimics the negative inotropic[4] and the inhibitory effect of acetylcholine on calcium influx during the cardiac action potential[5]. If cyclic GMP is an important metabolic intermediate in response to cholinergic stimulation, then the effects of acetylcholine should be enhanced if the breakdown of the cyclic nucleotide is inhibited. Papaverine is a potent inhibitor of both cyclic AMP and cyclic GMP phosphodiesterase activity in the rat heart[6] and has been shown to enhance the effects of catecholamines or derivatives of cyclic AMP in various heart muscle preparations[7]. In the present study, it will be demonstrated that also the inotropic effect of acetylcholine is enhanced in the presence of papaverine.

Materials and methods. Rats were anaesthetized with ether and left or right auricles were dissected from the heart and mounted in a muscle chamber for recording electrical and/or mechanical activity as described earlier[7,8]. Drugs (acetylcholine chloride and papaverine-HCl from Merck/Darmstadt, 8-bromo-cyclic GMP from Boehringer/Mannheim) were freshly dissolved in Tyrode's solution and injected into the muscle chamber containing 5 ml of Tyrode's solution (composition (mM): NaCl 136.9; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; NaH_2PO_4 0.42; $NaHCO_3$ 11.9; glucose 5.5) which was gassed with 95% O_2/5% CO_2 and kept at 35 °C. In the electrophysiological experiments, the bath was continuously perfused with Tyrode's solution with or without drugs.

Results and discussion. Figure 1 shows that the negative inotropic effect of acetylcholine was enhanced in the presence of papaverine. This finding supports the hypothesis that cyclic GMP is implicated in mediating the effects of cholinergic stimulation. The influence of papaverine on the inotropic effect of acetylcholine was assessed in rat left auricles electrically driven at 0.2 Hz. These experimental conditions have facilitated the evaluation of results. 1st, in contrast to other species, there is a negative staircase in rat hearts[9] (i.e., force of contraction is increased in a certain range when the driving rate is reduced). 2nd, the effects of acetylcholine are relatively weak at this frequency[10] and may be more susceptible to enhancing conditions. 3rd, there was virtually no effect of papaverine itself on the force of contraction if compared to the control values before addition of acetylcholine (n = 8). The negative inotropic effect of 10^{-4} M 8-bromo-cyclic GMP was not enhanced in the presence of papaverine (not shown). This does not conflict with the results obtained with acetylcho-

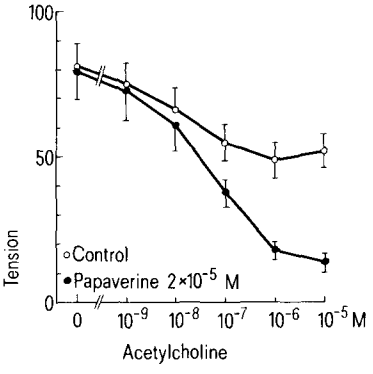

Fig. 1. Effects of acetylcholine on force of contraction (g/g w.w.t) of rat left auricles. ○, acetylcholine alone; ●, acetylcholine in the presence of papaverine 2×10^{-5} M. Symbols represent means±SE of 8 preparations. The preparations were first treated with acetylcholine alone and, after washing, the procedure was repeated in the presence of papaverine. Qualitatively the same results were obtained when the preparations were first tested in the presence of papaverine and then with acetylcholine alone.

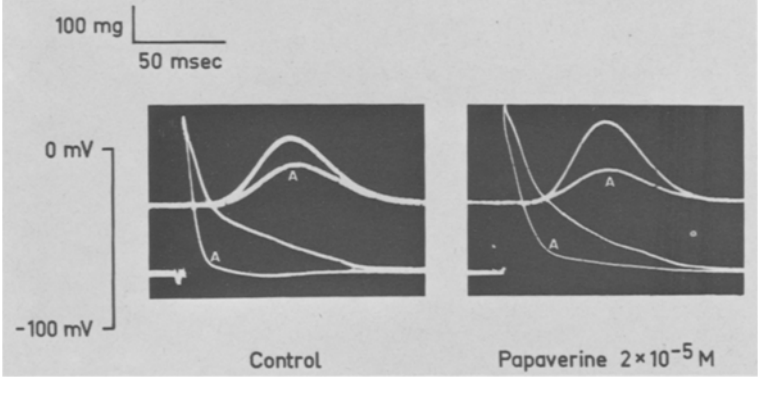

Fig. 2. Effects of acetylcholine 10^{-5} M on action potential configuration and force of contraction in a rat left auricle. Experimental procedure as described in figure 1. Records obtained with and without acetylcholine are superimposed, under control conditions (left) and in the presence of papaverine 2×10^{-5} M (right). In this experiment, papaverine itself produced increases in height and duration of the action potential and force of contraction. Acetylcholine (A) strongly reduced the action potential duration and depressed force of contraction in normal Tyrode's solution. In the presence of papaverine, the shortening effect of acetylcholine was less pronounced although the force of contraction was further depressed (to 110 mg with acetylcholine alone and to 82 mg in the presence of papaverine). Qualitatively the same results were obtained in 2 other experiments.

line, since 8-bromo-cyclic GMP is very resistant against degradation by phosphodiesterase activity[11] and the effect of this compound is rather limited by the cell membrane barrier. The enhancement by papaverine of the negative inotropic effect of acetylcholine is probably not due to the action of papaverine on the action potential, since the action potential is shortened by acetylcholine[12] and prolonged by papaverine[7]. Correspondingly, the shortening effect of acetylcholine on the action potential duration in rat atrial muscle is partially inhibited in the presence of papaverine, although the force of contraction is further depressed (figure 2).

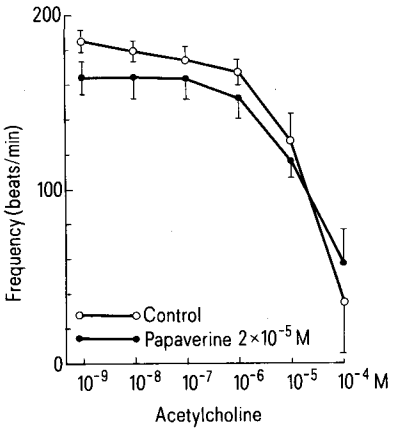

Fig. 3. Effects of acetylcholine on the frequency of spontaneously beating rat right auricles. ○, acetylcholine alone. ●, acetylcholine in the presence of papaverine 2×10^{-5} M. Symbols represent means±SE of 8 preparations. Experimental procedure as described in figure 1.

The pacemaker sensitivity of right auricles to acetylcholine was not significantly influenced in the presence of papaverine (figure 3). This result corresponds to the observation in rat hearts that 8-bromo-cyclic GMP did not influence the frequency of spontaneously beating right auricles[4] and would further suggest that cholinergic regulation of the pacemaker activity in rat right auricles is not modulated by cyclic GMP but rather by the effect of acetylcholine on the potassium permeability of the cell membrane[5].

1 I thank Lydia Paragnik for help. This research was supported by a grant from the Deutsche Forschungsgemeinschaft.
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Somatostatin reduces the release of colony-stimulating activity (CSA) from PHA-activated mouse spleen lymphocytes

W. Hinterberger, C. Cerny, H. Kinast, H. Pointner and K.H. Tragl[1]

1st Dept. of Medicine, University of Vienna, Lazarettgasse 14, A-1090 Wien (Austria), 7 November 1977

Summary. PHA-activated lymphocytes release colony-stimulating activity (CSA) for macrophage-granulocyte precursor cells (colony forming units, CFU_C) in the culture medium. Somatostatin, known to interfer with ribosomal protein synthesis, was demonstrated to reduce the release of CSA from PHA-treated mouse spleen lymphocytes.

Unspecific stimulation of lymphocytes induces blastogenesis, division and the release of lymphokines. The activity of a particular type of lymphokine, the colony-stimulating factor (CSF) is estimated by adding culture supernatants to human or mouse bone marrow agar cultures. The number of growing colony-forming cells (CFU_C) correlates with the colony-stimulating activity (reviewed by Metcalf[2]). The use of these techniques has produced evidence for different susceptibility for both DNA synthesis and release of CSF from mitogen-treated lymphocytes. Cytosin-arabinosid and vinblastin have been found to block DNA synthesis withouth blocking CSF release, while cycloheximide and puromycin inhibits CSF release without reduction of DNA synthesis[3].

Somatostatin, known to inhibit the release from the synthesizing cell of a spectrum of proteins was used in experiments instead of cycloheximide/puromycine. It was shown that activated mouse spleen lymphocytes cultured with somatostatin released less CSF into the culture medium than controls. Furthermore, DNA synthesis, as measured by 3H-TdR incorporation, was unaffected. The results described in this paper offer indirect evidence for interference of somatostatin with either the production or the release of CSF from PHA-activated mouse spleen lymphocytes.

Materials and methods. Spleen and bone marrow cells from C 57 bl mice (2-3 months old, both sexes, inbred) were used in all experiments. 3 mice were killed for each culture run and their femora were rinsed with Hank's solution. After 3 washes, bone marrow cells were suspended in McCoy 5 A medium containing 20% fetal calf serum. Spleen cells were suspended in cold RPMI 1640 medium following gentle dispersion through stainless steel sieves. Suspensions of mononuclear cells were obtained by Isopaque Ficoll gradient centrifugation (sp.wt 1077[4]). Adherent cells were removed by using the method of Messner[5]. Spleen cells were incubated in petri dishes for 1 h at 37 °C. Nonadherent cells were pipetted off and counted.

Table 1. Recovery of somatostatin in lymphocyte culture supernatants at various intervals. Mean values from 5 pooled supernatants (percentage of amount added)

1 h	24 h	48 h	72 h	96 h
68%	7%	6%	2%	0%