in the present study was that the parenteral feeding abolished the corticosterone rhythm. This was not due to a nonspecific effect of the liquid diet used, because rats fed on the same diet orally showed a clear rhythm corresponding to the feeding time. It seems also possible that the chronic i.v. infusion had some nonspecific effects on the adrenocortical activity, but this is not likely because the daily mean level of blood corticosterone was roughly equal in the orally and the i.v. fed rats. Therefore, oral feeding itself appears to be an important factor for establishing the corticosterone rhythm.

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A negative inotropic effect of acetylcholine in the presence of several phosphodiesterase inhibitors

H. Nawrath[1], P. Gruschwitz and X. Zong[2]

Department of Pharmacology, University of Mainz, D-6500 Mainz (Federal Republic of Germany), 9 October 1980

Summary. The phosphodiesterase inhibitors papaverine, theophylline and 3-isobutyl-1-methylxanthine (IBMX) reveal a negative inotropic effect of acetylcholine in cat ventricular heart muscle. This effect is unrelated to β-adrenoceptor stimulation and possibly mediated by the accumulation of cyclic GMP.

The regulation by cholinergic stimulation of the cyclic GMP content was first suggested by George et al.[3] in 1970. Since then, many reports in the literature have appeared which both support and vitiate the hypothesis that cyclic GMP may help to mediate the effects of acetylcholine (ACh) on the heart[4,5]. A 2nd messenger function of cyclic GMP in the heart is strongly validated if the physiological effects of ACh are enhanced when the breakdown of the cyclic nucleotide is inhibited. We report here that the phosphodiesterase inhibitors papaverine, theophylline and IBMX[6] reveal a negative inotropic effect of ACh in cat ventricular heart muscle which is normally not responsive.

Cats were anaesthetized with ether and papillary muscles or left and right atria were dissected from the heart and mounted in a muscle chamber for recording electrical and/or mechanical activity as described earlier[7]. The preparations were driven electrically at 0.2 or 1.0 Hz. Drugs were freshly dissolved and added to the muscle chamber containing Tyrode's solution (composition in 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 5.6) which was gassed with 95% O_2–5% CO_2 and kept at 35 °C. Isoprenaline in test solutions was protected from oxidative degradation by ascorbic acid (50 mg/l) and EDTA (18.6 mg/l).

Figure 1 shows that ACh 10^{-5} M exerted virtually no effects on the force of contraction in cat ventricular heart muscle. The concentration of ACh 10^{-5} M corresponds to an EC_{95} in cat atrial muscle in the same experimental conditions (n = 110; not shown). Figure 1 further demonstrates that the force of contraction in cat papillary muscles was increased to about twice the amount of the control in the presence of isoprenaline 3×10^{-7} M. When ACh was then added, the positive inotropic response to isoprenaline was diminished, yet less so in the presence of atropine 10^{-6} M. The antagonism between cholinergic and adrenergic stimuli in the ventricle is well known[8] and has been ascribed to a modulation by muscarinic receptors of adenylate cyclase activation[9]. It is in line with this interpretation that in the presence of both cholinergic and adrenergic stimuli the force of contraction in the ventricle is always above the control level. However, in the presence of papaverine or theophylline or IBMX the force of contraction can be depressed by ACh to values below the control, which reflects a direct negative inotropic action of ACh on ventricular heart muscle unrelated to β-adrenergic stimulation.

In the present study, papaverine was preferentially used, since the effects of this drug on the force of contraction are relatively small in cat ventricular heart muscle[7]. Papaverine is also known to have an effect on intracellular calcium being released from mitochondria[10]. However, independent of a positive or a negative inotropic effect of papaverine, a concentration-dependent negative inotropic effect of the drug was seen in the presence of ACh 10^{-5} M. This probably means that a graded inhibition of the phosphodiesterase activity reveals a negative inotropic effect of ACh on mammalian ventricular heart muscle which is

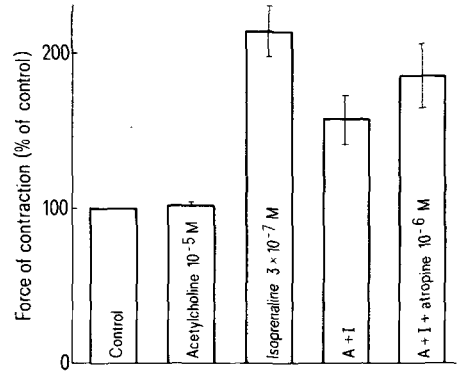

Fig. 1. Effects of ACh (A), isoprenaline (I), atropine and combinations of these drugs on the force of contraction in cat papillary muscles. After an equilibration period of 60–90 min the preparations were treated in the following sequence: 1. ACh 10^{-5} M for 15 min. 2. Drug-free Tyrode's solution for 30 min. 3. Isoprenaline 3×10^{-7} M (recording period 15 min). 4. Addition of ACh 10^{-5} M (recording period 15 min). 5. Addition of atropine 10^{-6} M (recording period 30 min). Frequency of stimulation: 1 Hz. Means ± SE of 8 preparations (paired data).

completely absent under normal conditions. Figure 2 depicts the concentration-response relationships of ACh in cat papillary muscles both under control conditions and in the presence of papaverine 10^{-4} M. An almost complete depression of the force of contraction was observed with increasing concentrations of ACh. In the presence of either theophylline 3×10^{-3} M or IBMX 3×10^{-4} M, the force of contraction was increased to $146\pm5.13\%$ (means $\pm$ SE; $n=8$) and $152\pm7.10\%$ ($n=8$) of control, respectively; upon the addition of ACh 10^{-5} M, the force of contraction was depressed to $63.7\pm6.53\%$ ($n=8$) and $55.6\pm3.4\%$ ($n=8$) of control, respectively (not shown). The negative inotropic effects of ACh were completely overcome in the presence of atropine 10^{-6} M or reversed by the removal of ACh from the bath fluid.

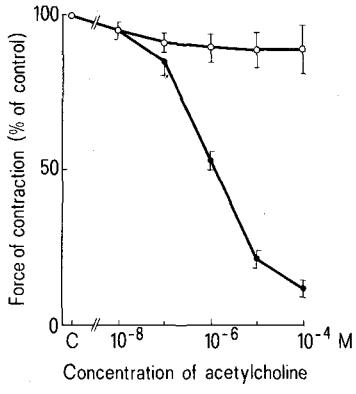

Fig. 2. Concentration-response relationships of ACh in cat papillary muscles without (○) and in the presence (●) of papaverine 10^{-4} M. After an equilibration period of 60–90 min the preparations were treated in the following sequence: 1. Cumulative addition of ACh; the effect of each concentration was observed for 10 min. 2. Drug-free Tyrode's solution for 30 min. 3. Papaverine 10^{-4} M (recording period 30 min). 4. Cumulative addition of ACh; the effect of each concentration was again observed for 10 min. Peak effects of ACh were evaluated. Frequency of stimulation: 1 Hz. Means $\pm$ SE of 12 preparations (paired data).

Figure 3 shows the effects of ACh, papaverine and the combination of both substances on the action potential configuration and the concomitant contraction. ACh alone had negligible effects on the force of contraction and the configuration of the action potential. Papaverine alone prolonged the action potential duration; this effect is due to a decrease by the drug of the potassium conductance at the cell membrane as shown by voltage clamp[11] and ^{42}K flux measurements[12]. In the presence of papaverine and ACh, the force of contraction was reduced to 37.3% of control. The duration of the action potential at 0 mV was reduced from 150 msec under control conditions to 110 msec. Similar findings were obtained in 6 further experiments. The effects of ACh on the action potential and the force of contraction were eliminated upon the addition of atropine 10^{-6} M (not shown).

The results shown here have 2 implications. a) There are muscarinic receptors in cat ventricular heart muscle which become functionally relevant when the activity of cyclic 3′:5′-nucleotide phosphodiesterase (EC 3.1.4.17) which catalyzes the hydrolysis of cAMP or cGMP to 5′AMP or 5′GMP[13] is inhibited. b) The direct negative inotropic effect of ACh on cat ventricular heart muscle is possibly related to the accumulation of cyclic GMP. Endoh and Honma[14] have shown that the accumulation of cyclic GMP in response to ACh is enhanced in the presence of papaverine. Furthermore, the negative inotropic effect of ACh in the ventricle is mimicked by 8-bromo-cyclic GMP[15].

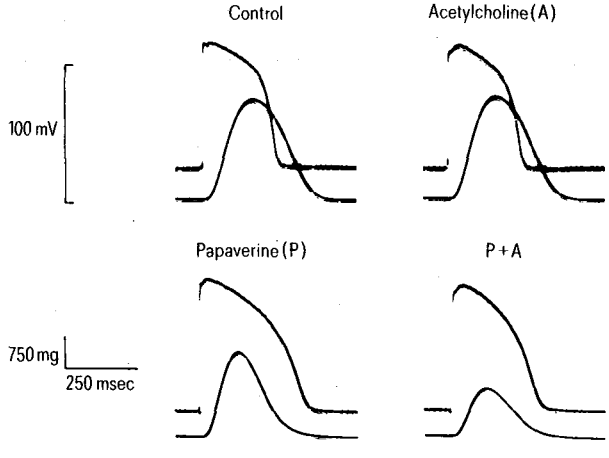

Fig. 3. Action potential and force of contraction of a cat papillary muscle under control conditions, in the presence of ACh 3×10^{-5} M, papaverine 2×10^{-5} M and in the presence of both substances. The preparation was treated in the same sequence as described in the legend to fig. 2. Frequency of stimulation: 0.2 Hz. Original records.

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2 Visiting scient from the Department of Pharmacology, Medical School of Wuhan, Wuhan, People's Republic of China.
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