Isoprenaline-like effects of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine on mechanical, biochemical and electrophysiological parameters in the mammalian heart[1]

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Summary. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) mimicked the effects of isoprenaline on the force of contraction, the cAMP content and the slow Ca^{++} inward current (I_{si}) in isolated guinea pig papillary muscles. The results support the hypothesis that phosphodiesterase inhibitors and β-adrenoceptor agonists exert their positive inotropic effects by increasing I_{si} via the common mediator cAMP.

Key words. 3-Isobutyl-1-methylxanthine; guinea pig heart; phosphodiesterase inhibitor; isoprenaline; slow inward current; positive inotropic effect; cAMP; cGMP.

It is generally accepted that the positive inotropic effect of β-adrenoceptor agonists is mediated by a stimulation of cardiac adenylate cyclase activity and a subsequent increase in cellular cAMP levels[3,4]. Cyclic AMP activates protein kinases, and this probably results in protein phosphorylation in the sarcolemmal slow Ca^{++} channel leading to an increased Ca^{++} inward current (I_{si})[5,6] and finally to an enhanced force development. Phosphodiesterase (PDE; EC 3.1.4.17) inhibitors are known to mimic, at least in part, the positive inotropic and cAMP elevating effects of catecholamines by inhibiting the hydrolysis of cAMP to 5'-AMP[3,4]. However, most of the PDE inhibitors have 'side effects' on e.g. Ca^{++} transport systems of the sarcoplasmic reticulum and therefore their positive inotropic and electrophysiological effects correspond only in part to their effects on cellular cAMP[7]. 3-Isobutyl-1-methylxanthine (IBMX) has been shown to be a potent phosphodiesterase inhibitor in a variety of tissues including guinea pig heart[8-11]. Since the effects of IBMX on isometric force of contraction closely resemble those of isoprenaline[12] IBMX is supposedly devoid of cAMP-independent 'side effects'. IBMX thus seems to be an 'ideal tool' to investigate whether inhibition of cAMP breakdown and stimulation of adenylate cyclase indeed lead to the same cardiac effects and to further substantiate that cAMP is the common mediator of the mechanical and electrophysiological effects of β-adrenoceptor agonists and PDE inhibitors in the heart. If this hypothesis holds true IBMX and the β-adrenoceptor agonist isoprenaline should similarly increase cAMP content and I_{si} in intact contracting heart muscle preparations. Therefore we studied the effects of IBMX on force of contraction, cAMP and cGMP content, and on normal and slow action potentials. Isoprenaline was studied for comparison.

Materials and methods. Guinea pigs (210–340 g b.wt) were pretreated with reserpine (Serpasil® ampoules Ciba; 5 mg/kg i.p.; 16–18 h before sacrifice) to prevent any interference from endogenously stored catecholamines. Papillary muscles (diameter 1 mm or less) were dissected from the right ventricles, attached to a platinum stimulating electrode and mounted individually in glass tissue chambers for recording the force of contraction as described previously[13]. The bathing solution (10 ml) containing (mmol l^{-1}) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 22.6, glucose 5.0, Na_2EDTA 0.05, ascorbic acid 0.28 was continuously gassed with 95% O_2 + 5% CO_2 and maintained at 35°C, pH 7.4. The preparations were electrically paced at 1 Hz with rectangular pulses of 5 ms duration; the voltage was 10–20% greater than threshold. For recording transmembrane potentials the guinea pig papillary muscles were mounted in a single sucrose-gap chamber of the type described by Beeler and Reuter[14]. The preparations were pulled through tightly fitting holes in two rubber membranes bounding a gap of 2 mm width. The preparations were also electrically paced at 1 Hz. Transmembrane potentials were measured with a conventional microelectrode technique[15]. For recording slow action potentials the potassium concentration was increased to 22 mmol l^{-1} without isotonic compensation in order to inactivate the fast sodium channels. Drugs used were 3-isobutyl-1-methylxanthine (IBMX; EgA-Chemie) and (±)-isoprenaline hydrochloride (Boehringer, Ingelheim). For mea-

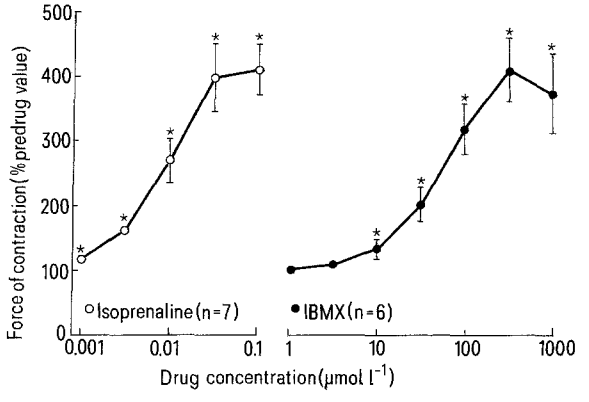

Figure 1. Concentration-response curves for the positive inotropic effects of isoprenaline (○) and 3-isobutyl-1-methylxanthine (IBMX, ●) in guinea pig papillary muscles. Ordinate: Force of contraction in percent of the predrug value. Abscissae: Drug concentration in μmol l^{-1}. The concentration-response curves were obtained cumulatively. The time of exposure to each concentration was 5 min for isoprenaline and 15 min for IBMX. The predrug values of force of contraction were 1.9 ± 0.2 mN (○; n = 7) and 1.8 ± 0.5 mN (●; n = 6). Asterisks denote significant differences from predrug values.

Table 1. Effects of 3-isobutyl-1-methylxanthine (IBMX) and isoprenaline on force of contraction, cAMP and cGMP content in isolated electrically driven guinea pig papillary muscles.

	cAMP content (pmol mg ww^{-1})	cGMP content (pmol mg ww^{-1})	Force of contraction (% predrug value)
Control	0.67 ± 0.03 (9)	0.032 ± 0.010 (8)	100.1 ± 2.2 (8)
IBMX 60 μmol l^{-1}	1.37 ± 0.16* (9)	0.085 ± 0.012* (6)	268.4 ± 22.9* (7)
Control	0.77 ± 0.04 (15)	0.017 ± 0.004 (15)	99.3 ± 1.5 (16)
Isoprenaline 0.01 μmol l^{-1}	1.23 ± 0.12* (12)	0.024 ± 0.006 (10)	295.0 ± 32.9* (10)

The incubation time was 15 min for IBMX and 5 min for isoprenaline. Control preparations received drug-free buffer alone. The predrug-values of force of contraction were 1.7 ± 0.3 mN (n = 15) and 1.4 ± 0.2 mN (n = 26) in the IBMX and isoprenaline series respectively. The numbers in parentheses give the number of experiments.
*$p < 0.05$ vs control.

Table 2. Effects of 3-isobutyl-1-methylxanthine (IBMX) and isoprenaline on normal action potential, slow action potential and force of contraction in isolated electrically driven guinea pig papillary muscles

Normal action potential	APD_{90} (ms)	APD_{20} (ms)	Plateau height (mV)	Amplitude (mV)	Resting potential (mV)	Force of contraction (mN)	n
Predrug value	250.6 ± 12.3	100.6 ± 5.5	19.0 ± 1.8	120.3 ± 3.5	− 86.8 ± 1.6	0.25 ± 0.02	
IBMX 60 µmol l^{-1}	217.5 ± 8.6*	109.7 ± 8.9	27.4 ± 2.6*	123.8 ± 3.6	− 87.1 ± 1.7	0.67 ± 0.09*	7
Predrug value	259.1 ± 23.5	112.0 ± 14.0	17.0 ± 3.5	117.6 ± 5.0	− 87.6 ± 2.7	0.25 ± 0.04	
Isoprenaline 0.01 µmol l^{-1}	229.1 ± 16.1*	116.0 ± 12.0	21.3 ± 4.5*	120.0 ± 5.3	− 87.8 ± 2.4	0.61 ± 0.09*	6

Slow action potential	APD_{90} (ms)	APD_{20} (ms)	dV/dt_{max} ($V\ s^{-1}$)	Amplitude (mV)	Resting potential (mV)	Force of contraction (mN)	n
Predrug value	152.5 ± 11.0	89.2 ± 5.1	3.5 ± 0.3	72.1 ± 4.2	− 50.0 ± 1.1	0.13 ± 0.01	
IBMX 60 µmol l^{-1}	207.1 ± 7.7*	113.2 ± 5.1	10.4 ± 0.9*	86.5 ± 3.5*	− 50.5 ± 0.8	0.41 ± 0.05*	7
Predrug value	135.5 ± 23.5	93.5 ± 10.8	2.8 ± 0.2	77.6 ± 2.0	− 50.9 ± 0.9	0.12 ± 0.01	
Isoprenaline 0.01 µmol l^{-1}	182.9 ± 17.7	109.4 ± 10.2*	6.8 ± 0.7*	84.0 ± 1.8*	− 51.2 ± 0.6	0.38 ± 0.05*	5

The parameters were measured before (predrug value) and 15 min (IBMX) or 5 min (isoprenaline) after drug addition. Action potential durations were determined at 20% and 90% repolarization (APD_{20} and APD_{90}). The plateau height was determined 50 ms after the onset of phase 0. Maximal rate of depolarization (dV/dt_{max}) was measured by electronic differentiation. n = number of experiments. *$p < 0.05$ vs control.

suring cAMP and cGMP content the preparations were removed from the organ bath and quickly frozen in liquid nitrogen[7]. cAMP and cGMP were measured by radioimmunoassays using the methods of Harper and Brooker[16] as described by Böhm et al.[17]. Recoveries run with each experiment amounted to 97.7 ± 2.9% (n = 35) for cAMP and to 100.0 ± 6.2% (n = 36) for cGMP and were not altered by the substances under investigation. Values presented are means ± SEM. Statistical significance was determined by Student's t-test. A p-value of less than 0.05 was considered significant.

Results and discussion. Figure 1 illustrates the concentration-response relationship for the positive inotropic effects of IBMX and isoprenaline. The positive inotropic effect of IBMX started at 10 µmol l^{-1} and maximally increased force of contraction to about 400% of the predrug value at 300 µmol l^{-1}. Isoprenaline increased force of contraction to the same extent, although much lower concentrations were needed (0.001–0.1 µmol l^{-1}). In the following experiments equieffective concentrations of IBMX (60 µmol l^{-1}) and isoprenaline (0.01 µmol l^{-1}) were chosen to compare the effects of both substances on cyclic nucleotide content and on electrophysiological parameters. From table 1 it is evident that the positive inotropic effects of both substances were accompanied by similar increases in cAMP content of the papillary muscles. IBMX also increased the cGMP content which was not affected by isoprenaline. Figure 2 illustrates the effects of the PDE inhibitor and the catecholamine on the shape of the normal and slow action potential. The positive inotropic effects of both substances were accompanied by a shortening of the normal action potential (APD_{90}) and an increase in plateau height (fig. 2; table 2; upper parts). The effects of IBMX and isoprenaline on slow action potentials (fig. 2; table 2; lower parts) evoked in potassium-depolarized papillary muscles were also similar. The action potential duration measured at 20% and 90% repolarization (APD_{20} and APD_{90}), the amplitude and the maximal rate of depolarization (dV/dt_{max}) were also increased to a similar extent by both substances.

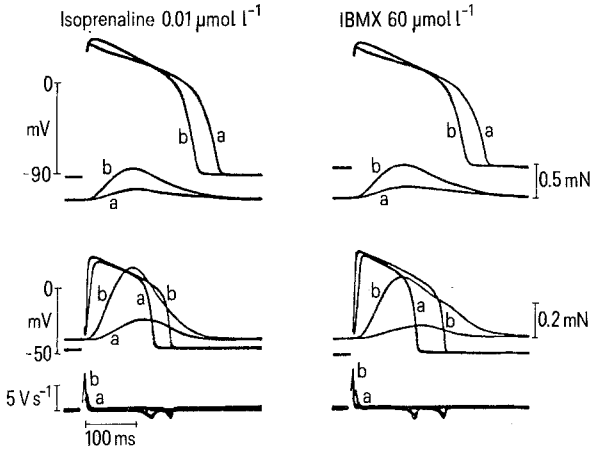

Figure 2. Effects of isoprenaline (left panels) and IBMX on normal action potentials (upper row) and slow action potentials (lower row) in electrically driven (1 Hz) guinea pig papillary muscles. Force of contraction was measured simultaneously.
Upper row: Normal action potentials (mV, upper traces) and force of contraction (mN, lower traces). Lower row: Slow action potentials (mV) and force of contraction (mN, upper traces); maximal rate of depolarization (V s^{-1}, lower traces). Slow action potentials were elicited after raising the potassium concentration from 5.4 to 22 mmol l^{-1}.
a Control; *b* Isoprenaline 0.01 µmol l^{-1} (5 min) or IBMX 60 µmol l^{-1} (15 min) resp.

The results demonstrate that the PDE inhibitor IBMX effectively mimicked the positive inotropic and the cAMP-elevating effect of the β-adrenoceptor agonist isoprenaline. Moreover, its effects on the electrical activity of the heart also were very similar to those of isoprenaline. The increase in plateau height of the normal action potential which is assumed to reflect an increase in I_{si}[18] indicates that both substances finally exert their positive inotropic effect by increasing I_{si}. More direct evidence for this view can be deduced from the increase in dV/dt_{max} of the slow action potentials. Since slow action potentials are evoked in preparations in which the fast Na^+ inward current is inactivated by depolarization, phase 0 of these responses is predominantly carried by Ca^{++} and dV/dt_{max} can be taken as a measure of I_{si}[19]. The results are in accord with previous findings that effects of phosphodiesterase inhibitors on the shape of the action potential and on I_{si} are similar to those of adrenaline and noradrenaline in calf Purkinje fibers[20–22]. Thus, the present results support the hypothesis that PDE inhibitors and

β-adrenoceptor agonists both exert their positive inotropic effects by increasing I_{si} via the common mediator cAMP. In accord with this IBMX and isoprenaline have previously been shown to similarly shorten the time to peak force and the relaxation time of the isometric contraction, as cAMP elevating drugs characteristically do[12,23]. These previous findings are not contradicted by the fact that in the electrophysiological experiments presented here the shortening of the total duration of the contraction was but marginally pronounced, if at all. With the single sucrose-gap chamber used the preparations are fixed with a rubber membrane so that the relaxation of the contraction is very slow and changes in relaxation time are barely detectable (see also Reuter[18]).

There was only one point in which the effects of isoprenaline and IBMX differed. IBMX increased the cGMP content while isoprenaline did not. The IBMX-induced increase in cGMP content is probably due to the inhibition of a cGMP phosphodiesterase[24]. However, since the effects of IBMX and isoprenaline on force of contraction and on normal and slow action potentials were almost the same, we conclude that cGMP does not play an important role in mediating mechanical and electrophysiological effects of IBMX in the heart.

1 Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft.
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0014-4754/85/060732-02$1.50 + 0.20/0

Effects of oral contraceptive steroids (norethisterone/mestranol) on the activities of hepatic drug-metabolizing enzymes in iron-deficient anemic rats[1]

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Summary. Either oral contraceptive steroid (norethisterone/mestranol; N/M) treatment or iron-deficiency (Fe(–)) anemia alone caused an increase in NADPH cytochrome c reductase and in three hepatic microsomal mixed-function oxidase activities in female rats. When N/M treatment and the Fe(–) diet are combined, no further change in hepatic enzyme activity is seen compared with that with either treatment alone.
Key words. Drug metabolism; iron-deficient anemia; norethisterone/mestranol; oral contraceptive steroid.

The pharmacokinetics of many drugs have been shown to be modified following the induction of increased activity of the microsomal mixed-function oxidase (MFO) and other hepatic enzyme systems. Among these inductive factors are oral contraceptive steroids (OCS)[4] and iron-deficiency (Fe(–)) anemia[5]. In view of the frequency with which OCS treatment and Fe(–) anemia coexist in women of child-bearing age, it is important to evaluate the effect of OCS treatment on drug metabolism in the presence of such anemia. To this end, we have studied the variations of the activities of the microsomal MFO and the electron transport system in the liver of female rats treated with OCS (norethisterone/mestranol; N/M) and a Fe(–) diet, either singly or together. Data on the changes of hepatic metalloenzymes, serum lipids, and aortic glycosaminoglycan levels under the same experimental conditions have been reported previously[6,7].

Methods. Female Wistar rats weighing approximately 50 g were randomly divided into four groups of 20 animals. The control group was fed a normal diet throughout the 8-week experimental period; the N/M treated group was fed the normal diet for the first 4 weeks and then the N/M containing normal diet for an additional 4 weeks; the Fe(–) anemia group was fed the Fe(–) diet over a period of 8 weeks; and the last group for the combination study was fed the Fe(–) diet for the