Vol. 167, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1990 Pages 123-129

DIFFERENTIAL EFFECTS OF RO 20-1724 AND ISOBUTYLMETHYLXANTHINE ON THE BASAL FORCE OF CONTRACTION AND β-ADRENOCEPTOR-MEDIATED RESPONSE IN THE RAT VENTRICULAR MYOCARDIUM

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Received December 6, 1989

Effects of Ro 20-1724, a selective inhibitor of soluble cGMP-insensitive type IV phosphodiesterase, on the force and cAMP levels were compared with those of 3-isobutyl-1-methylxanthine, a non-selective inhibitor, in the rat ventricular myocardium. Ro 20-1724 scarcely affected the basal force of contraction and cAMP levels, whereas it enhanced the positive inotropic effect and cAMP accumulation induced by isoproterenol more effectively than 3-isobutyl-1-methylxanthine. These results imply that inhibition of the soluble cGMP-insensitive type IV PDE by Ro 20-1724 may be crucially involved in the regulation of myocardial contractility through the interaction with cAMP generation in the rat ventricular myocardium.

Non-selective inhibition of cyclic nucleotide phosphodiesterase (PDE) by classical PDE inhibitors such as theophylline and 3-isobutyl-1-methylxanthine (IBMX) results in both an increase in basal force of contraction (the direct positive inotropic effect) and an enhancement of the β-adrenoceptor-mediated positive inotropic effect (the enhancing effect) in cardiac muscle. More recently it became evident that selective inhibition of cAMP specific (with low K_m) cGMP-inhibitable type IV PDE (more commonly termed FIII or peak III PDE) in the particulate fraction of homogenates of cardiac muscle cells is critical for induction of direct positive inotropic effects of newly developed cardiotonic agents such as amrinone, milrinone, enoximone, piroximone, imazodan and indolidan (1-7). This PDE isozyme shows a wide range of variations among mammalian myocardium, and the rat cardiac muscle that lacks particulate type IV isozyme does not respond to these newly developed compounds (5). On the other hand, another subclass of agents, Ro 20-1724 and rolipram, that inhibits selectively the cGMP-insensitive type IV PDE in the soluble fraction has been shown to be ineffective to elicit the direct positive inotropic effect in the mammalian myocardium examined (8,9). Selective inhibition of type I or type II PDE has likewise been shown to be uninvolved in the regulation of myocardial

0006-291X/90 $1.50

contractility (9,10). During the course of investigation of inotropic and cAMP-accumulating effects of PDE inhibitors in our laboratories, we found that Ro 20-1724 is more effective than IBMX to enhance the β-adrenoceptor-mediated positive inotropic and cAMP-accumulating effects in the rat ventricular myocardium. This implies that different types of PDE isozymes may be involved in the regulation of cAMP metabolism in the direct positive inotropic and enhancing effects on the β-mediated response and/or species difference of PDE isozymes may affect the induction of both effects in ventricular myocardium of different mammalian species.

MATERIAL AND METHODS

The isolated rat right papillary muscles and cardiac myocytes were used for determinations of the drug effects on force of contraction and cAMP levels, respectively.

Isolated right papillary muscle. Male Wistar rats (300-400 g) were lightly anesthetized with ether. The right ventricular papillary muscles were isolated and mounted in 20 ml organ baths containing Krebs-Henseleit solution [Na^+ 142.9, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 1.25, H_2PO^{4-} 1.2, HCO_3^- 24.9, SO_4^{2-} 1.2, Cl^- 127.8 and glucose 11.1 (mM)] bubbled with 95% O_2 and 5% CO_2 at temperature of 37 ± 0.5°C (pH 7.4). Muscles were electrically stimulated by square wave pulses of 5 ms-duration and a voltage about 20% above the threshold at 1 Hz. Force of isometric contractions was recorded on a thermal pen writing oscillograph (Recti-Horiz-8K, NEC-San-ei, Tokyo, Japan) by means of strain gauge transducers (UL-10GR Shinkoh Communication Industry Co., Tokyo, Japan). After an equilibration period of 1 h, (±)-bupranolol (10^{-6} M) was added into the bath to eliminate the influence of endogenous norepinephrine on the direct effect of the test drug. Bupranolol at this concentration did not affect the basal force of contraction. Ro 20-1724 and IBMX were administered in a cumulative manner to investigate the direct effect. Experiments for detection of enhancing effects of the compounds on the β-mediated response, the concentration-response curves for isoproterenol were determined successively in the absence and presence of Ro 20-1724 or IBMX, respectively. The PDE inhibitors were allowed to act for 10 min prior to determination of the second concentration-response curve for isoproterenol. These experiments were carried out in the absence of bupranolol.

Determination of cAMP in isolated ventricular myocytes. Male Wistar rats (190-230 g) were heparinized (500 U i.p.) and lightly anesthetized with ether. Hearts were quickly removed and perfused with Krebs-Henseleit solution containing collagenase (70 U/ml) and 0.1% bovine serum albumin (BSA) in the presence of 50 μM Ca^{2+} according to the method of Powell and Twist (11). The viability of the freshly prepared cells was about 90%. Incubation of cardiomyocytes and extraction of cAMP from the cells were performed according to the method described by Katada and Ui (12) with a slight modification. Briefly, each incubation tube contained 100 μl of cells (5 x 10^5 cells/ml) suspended with Krebs-Henseleit solution containing 2% BSA and 100 μl of physiological saline with drugs. Incubation was carried out at 37°C. At the end of incubation, 200 μl of 0.2 N HCl was quickly added and the tube was immersed in boiling water for 3 min to extract cAMP. After centrifugation at 600 x g for 10 min, cAMP was assayed in duplicate by the sensitive radioimmunoassay method (Yamasa Shoyu Co., Choshi, Japan). Ro 20-1724 and IBMX were incubated with the suspension of myocytes for 5 min prior to administration of isoproterenol. The cAMP levels were determined 2 min after adding isoproterenol in the absence or presence of the cAMP-PDE inhibitors. The vehicle of Ro 20-1724 and IBMX had no effects on the cAMP level.

Materials. Drugs were obtained from the following sources: Ro 20-1724 was generously supplied from Hoffman-La Roche Inc. (Nutley, NJ, USA); 3-isobutyl-1-methylxanthine (Aldrich Chemical Co., Milwalkee, WI, USA); (-)-isoproterenol hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); (±)-bupranolol hydrochloride (Kaken Kagaku, Tokyo, Japan). Ro 20-1724 and IBMX were dissolved in 95% ethanol and 50% N,N-dimethylformamide, respectively, in a concentration of 2 x 10^{-4} M. These drugs were diluted to the desired concentration with physiological saline solution.

Statistical evaluation. Experimental values were presented as means ± S.E.M. Statistical significance between mean values was estimated by Student's t-test. A t-test for the paired comparison was used when it was applicable. A p value smaller than 0.05 was considered to be significant.

RESULTS

Direct effects of Ro 20-1724 and IBMX. IBMX elicited a positive inotropic effect in a concentration-dependent manner at 10^{-5} M and higher, while Ro 20-1724 scarcely affected the force of contraction (Fig. 1). The positive inotropic effect of IBMX reached a steady level within 5-10 min after the administration.

The concentration-effect curves for cAMP accumulation by Ro 20-1724 and IBMX are shown in Fig. 2. The cAMP content was determined at 2 min after the administration of the compounds, since the cAMP level reached a steady level at 2 min after adding IBMX. IBMX elevated markedly the cAMP level depending on the concentration. Ro 20-1724 (10^{-6} - 10^{-4} M) did not

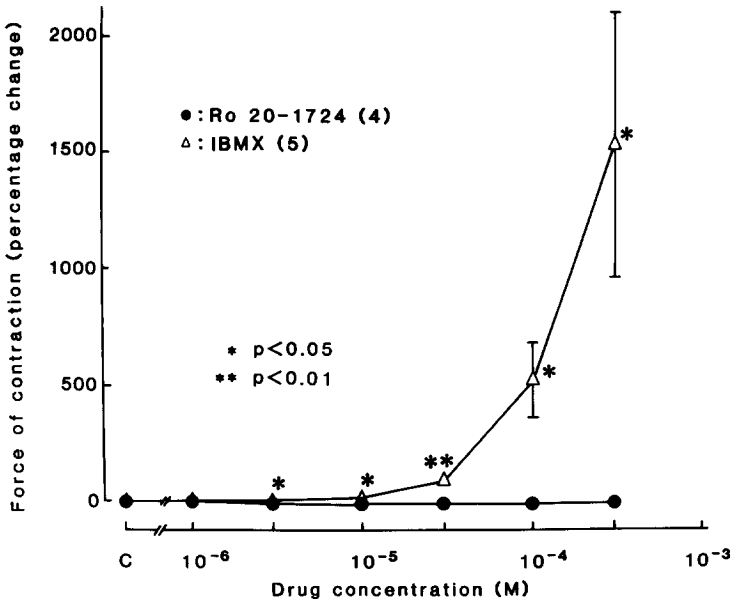

Figure 1: Concentration-response curves for Ro 20-1724 and IBMX to increase the force of contraction in the isolated rat right papillary muscle. Numbers of experiments in parentheses; vertical lines: S.E.M. *Significantly different vs. the respective control values. Bupranolol (10^{-6} M) was present throughout the experiments.

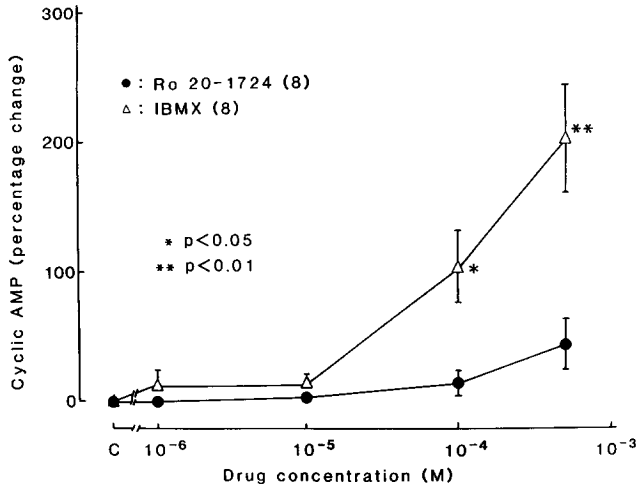

Figure 2: The concentration-effect curves for Ro 20-1724 and IBMX to accumulate cAMP in the isolated rat cardiomyocytes. Basal values for cAMP contents: 1.26 ± 0.12 pmol/10^5 cells (Ro 20-1724) and 1.06 ± 0.08 pmol/10^5 cells (IBMX). Numbers of experiments in parentheses; vertical lines: S.E.M. *Significantly different vs. the respective control values.

affect the cAMP level; at 5 x 10^{-4} M Ro 20-1724 slightly elevated the level, the increase being not significant.

Influence on isoproterenol-induced positive inotropic and cAMP-accumulating effects. Effects of Ro 20-1724 and IBMX on the concentration-response curve for the positive inotropic response to isoproterenol are shown in Fig. 3. The concentration-response curve for isoproterenol was shifted to the left and upward by Ro 20-1724 (10^{-5} M), while Ro 20-1724 at this concentration did not affect the basal force of contraction (Fig. 3a). The pD_2 value (-log EC_{50}) for isoproterenol was significantly increased by Ro 20-1724 from the control value of 7.10 ± 0.14 to 7.44 ± 0.11 (n = 6, each). IBMX (10^{-5} M) shifted likewise the concentration-response curve for isoproterenol to the left. The pD_2 value for isoproterenol was significantly increased from 7.24 ± 0.19 to 7.97 ± 0.19 (n = 6, each).

Effects of Ro 20-1724 and IBMX on the concentration-effect curve for accumulation of cAMP induced by isoproterenol are shown in Fig. 4. In the absence of the cAMP-PDE inhibitors isoproterenol elevated the cAMP level in a concentration-dependent manner, the maximal level (306 ± 60%; control cAMP level, 1.36 ± 0.12 pmol/10^5 cells; n = 8) being achieved at 10^{-5} M. The concentration-response curve for isoproterenol was markedly shifted upward in the presence of Ro 20-1724 or IBMX. The former was much more effective than the latter in enhancing the isoproterenol-induced accumulation of cAMP. The maximal cAMP levels in response to isoproterenol in the presence of Ro 20-1724 and IBMX were 2336 ± 411% (control level with Ro 20-1724,

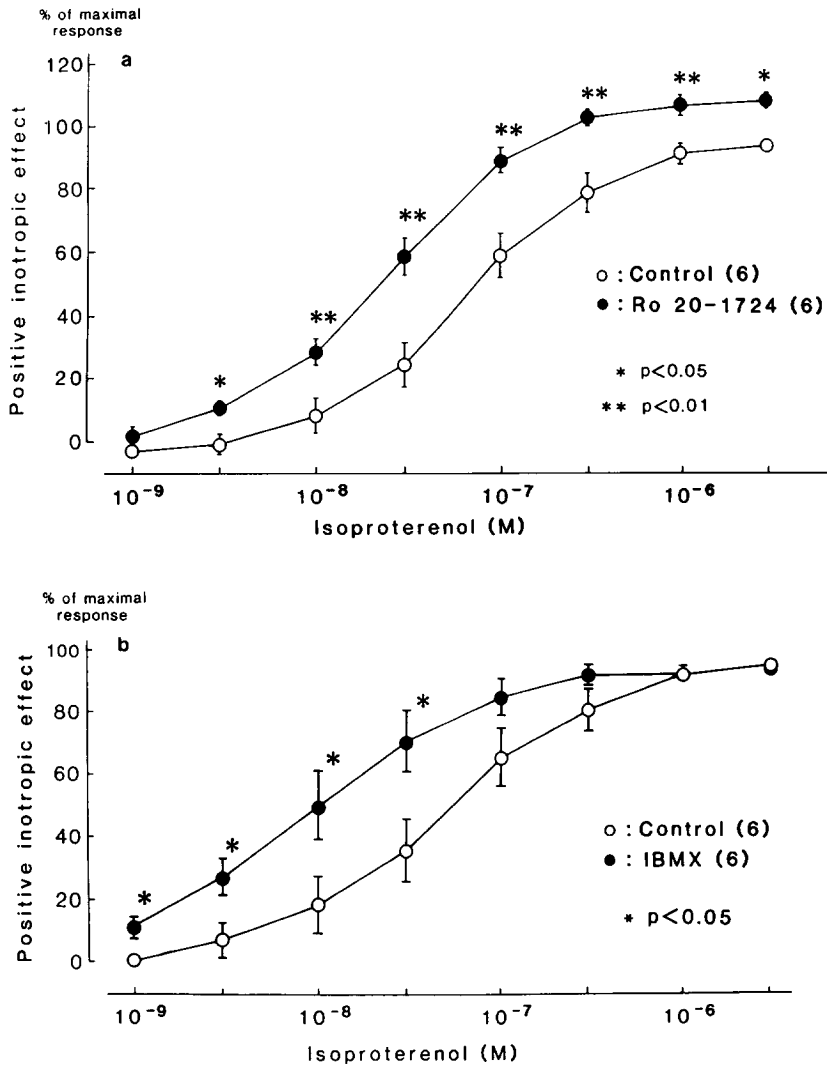

Figure 3a and b: Influence of Ro 20-1724 (10^{-5} M) in **a** and IBMX (10^{-5} M) in **b** on the concentration-response curve for isoproterenol to increase the force of contraction in the isolated rat right papillary muscles. The maximal response to isoproterenol in the first determination was taken as 100%. Numbers of experiments in parentheses; vertical lines: S.E.M. *Significantly different vs. the respective control values.

1.50 ± 0.20 pmol/10^5 cells; n = 6) and 842 ± 215% (control cAMP level with IBMX, 1.90 ± 0.32 pmol/10^5 cells; n = 6), respectively.

DISCUSSION

Since long it has been postulated that enzymes involved in the metabolism of cAMP may be localized unevenly in the membranes of sarcolemma and

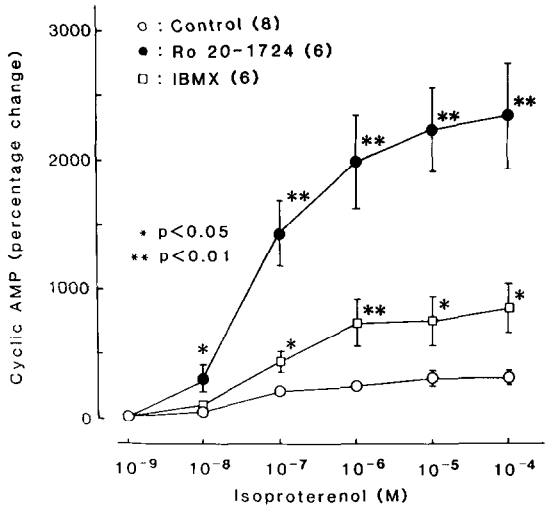

Figure 4: Influence of Ro 20-1724 and IBMX on the concentration-effect curve of isoproterenol-induced accumulation of cAMP in the isolated rat cardiomyocytes. The cAMP levels were determined 2 min after adding isoproterenol. The cAMP-PDE inhibitors were allowed to act for 5 min prior to isoproterenol. Numbers of experiments in parentheses; vertical lines: S.E.M. *Significantly different vs. the respective control values.

sarcoplasmic reticulum (SR). This may result in localized accumulation of cAMP in the intracellular compartment. Although the experimental evidence is still fragmental, this hypothesis has been supported by good pieces of evidence obtained by refined fractionation of cellular components after homogenization of myocardial cells. Prenalterol, a β_1-adrenoceptor partial agonist, has recently been shown to accumulate cAMP selectively in the particulate fraction (100,000 x g), which is correlated well with the increase in force of contraction, while isoproterenol causes an additional marked cAMP accumulation in the soluble fraction (13). As concerned with the cAMP-PDE inhibitors it is demonstrated that the selective inhibition of type IV PDE in the particulate fraction is relevant to the direct positive inotropic effect of newly developed cardiotonic agents (see Introduction for Refs.). In the present study we showed in the rat ventricular myocardium that the selective inhibition of soluble cGMP-insensitive type IV PDE by Ro 20-1724 may be involved in the regulation of myocardial contractility under the interaction with β-adrenoceptor activation. The observation that Ro 20-1724 is more effective than a non-selective cAMP-PDE inhibitor IBMX is unexpected and the subcellular mechanism underlying remains unsolved in the present study. In order to get insight into the cAMP metabolism in locally restricted intracellular compartment, we have to take into consideration both the inhomogenous distribution of cAMP-PDE isozymes and PDE inhibitors, but the experimental evidence pertinent to these informations is lacking at the present time. Considering that the β-adrenoceptor-adenylyl cyclase sys-

tem exists in sarcolemma, generation of cAMP may be promoted more readily in the vicinity of cell membrane. Therefore, it appears to be reasonable to suppose that the inhibition of type IV isozyme in soluble fraction may be more effective than that of type IV in the particulate fraction mostly composed of SR membranes. This assumption is supported by our recent findings in the same experimental system that milrinone, an inhibitor of the latter (14), caused a direct positive inotropic effect in association with an accumulation of cAMP, but did not affect the concentration-response and concentration-effect relations for isoproterenol (15).

In summary, the present findings imply that the inhibition of soluble cGMP-insensitive type IV PDE by Ro 20-1724 alone in the rat ventricular myocardium does not contribute to facilitate the myocardial contraction, while it may come into play an important role in the facilitatory regulation of myocardial contractility under sympathetic excitation in vivo through potentiation of the positive inotropic and cAMP generating effects via β-adrenoceptor activation.

ACKNOWLEDGMENTS: This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (No. 62624004 and 63641002) from the Ministry of Education, Science, and Culture, Japan, and Uehara Memorial Foundation.

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